DEVELOPMENT OF PARENTERAL DRUGS FOR THE TREATMENT OF SEIZURE EMERGENCIES

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DEDICATION

To all patients affected by seizure emergencies

&

To my teachers for their endless patience and confidence in me

&

To my family and friends for their unconditional love and support

ABSTRACT

The overall **objective of my dissertation is to develop alternative therapies for seizure emergencies**. Status epilepticus is a condition defined as a convulsive seizure lasting more than 5 minutes and is considered a seizure emergency due to the increased risk for neuronal damage and mortality (Trinka et al. 2015). Although relatively effective, first-line therapy fails to terminate status epilepticus in 26-57% of cases, leading to increased risk of seizure refractoriness and use of second- and third-line therapies that may increase the risk of systemic complications and mortality (Treiman et al. 1998; Alldredge et al. 2001; Silbergleit et al. 2012; Chamberlain et al. 2014). Three drugs were studied: allopregnanolone (ALLO), lacosamide (LCM) and topiramate (TPM). The pharmacokinetics and pharmacodynamics of investigational allopregnanolone formulations following intravenous and intramuscular delivery were assessed for the development as an early rescue therapy for seizure emergencies (Project 1). I also explored the relationship between lacosamide and PR prolongation in the critically-ill population to identify a subpopulation in whom it can be used safely (Project 2). Finally, for topiramate, the pharmacokinetics and pharmacodynamics of an investigational intravenous formulation was evaluated for adjunctive therapy in seizure emergencies (Project 3).

Allopregnanolone, a progesterone derivative and $GABA_A$ positive allosteric modulator, has demonstrated potential to treat status epilepticus in preclinical models and pediatric and adult patient case reports. Given that first-line therapy fails in the majority of cases, more effective early treatments are necessary to

prevent downstream seizure refractoriness and systemic complications. The specific aims for Project 1 were to characterize the pharmacokinetics, pharmacodynamics and safety following intravenous and intramuscular ALLO in dogs. Five dogs (one on phenobarbital therapy) received single doses of ALLO: one- to four-mg/kg intravenously, or one- to six-mg/kg intramuscularly, with a washout period of at least one week. Plasma samples were collected pre-dose and at regular intervals up to six hours post-dose. Clinical response was assessed by behavioral response and intracranial electroencephalographic (iEEG). I found that with IV ALLO, drug exposure and peak plasma concentration increased proportionally with dose within the doses studied. Behavioral responses and iEEG data illustrate the rapid onset of effect following IV ALLO administration. The results of this study indicate that IV ALLO is a promising agent for the early treatment of seizure emergencies, with evidence of rapid penetration into the brain and a high safety profile. IM ALLO has great potential to be useful as a first-line treatment for SE, but the current formulations do not attain high enough plasma concentrations predicted to confer iEEG changes. Therefore, alternative approached would be needed for a viable IM ALLO product.

Intravenous LCM has shown safety and some efficacy as an adjunctive therapy in refractory convulsive and non-convulsive status epilepticus. Outside of seizure emergencies, it is also used in the critically-ill population to treat acute breakthrough seizures or to maintain seizure control in patients who are unable to take oral medications. Lacosamide is particularly appealing in this patient

population due to its low potential for drug-drug interactions and serious systemic complications. However, there are reports of PR interval prolongation, which raises concern for patients who have a higher risk for developing cardiac arrhythmias or conduction abnormalities. The specific aim of Project 2 was to estimate the prevalence of PR prolongation in the critically-ill patient population following intravenous LCM administration. I performed a retrospective chart review and defined PR interval prolongation as a shift from normal to high PR interval or an increase of 20% or more in PR interval from baseline. Logistic regression analysis was performed in order to identify clinical factors that help predict an increase in PR interval ≥20%. Eight percent of my patient sample experienced PR prolongation, which is 20-times higher than the prevalence of 0.4% reported in ambulatory patients with epilepsy. The logistic regression analysis suggested that the occurrence of PR prolongation following IV LCM administration is positively associated with age, the total daily dose of LCM, and serum potassium levels. However, considering that these results are generated from a small number of events (n=7/88), the true impact of these predictors on PR prolongation in this patient population needs to be explored further.

In addition to finding alternative early treatments for seizure emergencies, better adjunctive treatments during refractory stages of status epilepticus are also needed. Topiramate's many mechanisms of action and preclinical evidence of neuroprotection, which make it an ideal candidate to treat status epilepticus that has become resistant to first-line therapies. Intravenous administration of TPM offers an alternative that would allow more drug to get into the body and at

a faster rate than current methods of its administration. The specific aims of Project 3 were to characterize the pharmacokinetics, pharmacodynamics and safety following intravenous TPM in dogs. Five dogs (three on phenobarbital maintenance therapy) were used in this study. Ten and twenty mg/kg of stable-labeled topiramate were infused intravenously over five minutes. One hour following the 10 mg/kg infusion, each dog also received a 5 mg/kg dose of unlabeled oral topiramate. Plasma samples were collected pre-dose and at regular intervals up to nine hours post-dose. Sixteen electrode channels were continuously recorded. Topiramate concentration-time data were analyzed using noncompartmental and population compartmental approaches. Concentration-time data were best fit by a two-compartment model, and co-medication with phenobarbital was associated with a 5.6-fold higher clearance. The estimated absolute oral bioavailability ranged from 62-102%. Statistically significant increases in iEEG activity were observed within 30 minutes of infusion, which is essential when treating seizure emergencies. Simulations suggest a different dosing strategy for dogs on phenobarbital may be necessary to optimize drug exposure. The results of this study indicate that development of an intravenous TPM formulation with evidence of penetration into the brain and good tolerability is feasible.

My research suggests that there are promising therapies in development for the management of SE, which will significantly improve patient lives by offering safer use of current antiseizure drugs or more effective therapies. There are many pathways into which these projects can take, including conducting clinical

trials in dogs with naturally-occurring SE and single- and multiple-ascending dose studies in patients.

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CHAPTER 1

INTRODUCTION

1.1 Introduction and Orientation

Status epilepticus (SE) is a life-threatening condition that requires rapid treatment in order to prevent systemic complications and irreversible brain damage. Although there are evidence-based guidelines for the management of convulsive SE, including the use several antiseizure drugs with different mechanisms of action, the case fatality rate within 30 days of the SE event ranges from 21-39% (Logroscino et al. 1997; Vignatelli, Tonon, and Alessandro 2003).

As the duration of a seizure lengthens, the seizure becomes less likely to terminate on its own and more difficult to treat with current therapies (J. W. Chen and Wasterlain 2006; Fujikawa 1996; Mazarati, Baldwin, et al. 1998). After 30 minutes of prolonged seizure activity, the risk for neuronal cell damage escalates. Therefore, time is essential in the management of SE, and rapid intervention with the goal of seizure termination is key. Even with relatively effective first-line therapies, roughly 30% of cases fail to respond and progress to more serious conditions (Treiman et al. 1998). The current treatment of SE is suboptimal, especially earlier on in treatment algorithm. There is a need for safe and effective alternatives to better manage this condition.

In general, the rapid intervention of SE is determined by the routes of drug administration. For this condition, the ideal intervention is one that can be administered with ease and achieve therapeutic drug concentrations in the brain within a short amount of time. For this to be possible, the drug must be able to be

formulated in a solution that allows for intravenous and depending on its physicochemical properties, intramuscular, intranasal or subcutaneous administration. While enteral and rectal routes of administration have been used for SE treatment, they are limited by slower rates of absorption (and consequently lower and delayed peak drug concentrations) and decreased social acceptance, respectively (Bhattacharyya, Kalra, and Gulati 2006; Brigo et al. 2015). Thus, in this dissertation, the focus will be on parenteral formulations of central nervous system-active (CNS-active) drugs.

The overarching **objective of my dissertation is to develop alternative therapies for seizure emergencies**, which will have a significant impact on the patients and families of those affected by offering safer use of current treatments or more effective treatments. As part of this work, I will present a review of human epilepsy and SE, followed by canine epilepsy and SE, and the translatability of therapeutic and mechanistic research between the two diseases. Although my primary focus is on treatment alternatives for SE and not the management of epilepsy syndromes, it is essential to understand the underlying pathophysiology of seizures and epileptogenesis before attempting to treat prolonged seizures. The prospective therapies under development range in their stages in the drug development pipeline, as well as their potential place in the management of seizure emergencies. These include:

- Project 1: Allopregnanolone, a naturally-occurring neurosteroid that is a positive allosteric modulate $GABA_A$ receptors, with potential as an early treatment of SE
    - Hypothesis: Allopregnanolone would be beneficial in the early treatment of SE based on its novel mechanism of action and ability to get into the brain quickly
    - **Specific aim: To characterize the pharmacokinetics, pharmacodynamics, and safety/tolerability following intravenous and intramuscular allopregnanolone in dogs**
- Project 2: Lacosamide, an antiseizure drug that enhances the slow inactivation of voltage-gated sodium channels, with potential as a treatment for established SE but has concerns for cardiac safety
    - Hypothesis: Intravenous lacosamide increases the risk for PR prolongation, especially in the critically-ill population
    - **Specific aim: To estimate the prevalence of PR prolongation in the critically-ill patient population following intravenous lacosamide administration**
- Project 3: Topiramate, an antiseizure drug that potentiates GABA current and antagonize AMPA/kainite receptors, with potential as an adjunctive treatment for refractory SE
    - Hypothesis: Intravenous topiramate would be beneficial as an adjunctive treatment for refractory SE based on its multiple mechanisms of action and low potential for drug-drug interactions

- **Specific aims: To characterize the pharmacokinetics, pharmacodynamics, and safety/tolerability following intravenous topiramate in dogs**

As part of the review, I will refer to ratings of evidence and levels of recommendation which are categorized based on systematic reviews conducted by Glauser et al 2016 and Podell et al 2016. In general, these authors rate evidence depending on the type of clinical studies conducted. High level of evidence is from a prospective, blinded, randomized, controlled clinical trial (RCT) with masked outcome assessment in a representative population. Moderate level of evidence is from a prospective randomized matched group cohort study. Low level of evidence is from uncontrolled studies, case series, case reports, or expert opinion. Consequently, the level of recommendation for specific therapies are based on the level of evidence available for an indication. For example, a high recommendation is given if treatment is established with high level of evidence as effective and should be given, while a moderate recommendation is given if the treatment is probably effective and should be considered (Glauser et al. 2016).

1.2 Human Epilepsy and Seizure Emergencies

1.2.1 Human Epilepsy

Epilepsy is a disease of the brain that is characterized by the presence and/or predisposition for seizures. An epileptic seizure is a passing occurrence of

symptoms due to abnormal electrical brain activity (Fisher et al. 2005). The International League Against Epilepsy (ILAE) is an organization founded in 1909 whose goals are to advance the knowledge of epilepsy, promote its research and education, and improve the care of patients with epilepsy (About International League Against Epilepsy 2019). As part of their mission, the ILAE is tasked with defining and classifying seizures and epilepsy. In 2014, a practical definition of epilepsy was established to aid in the diagnosis of the disease. Epilepsy is diagnosed by the presence of any of the following: “1) at least two unprovoked (or reflex) seizures occurring more than 24 hours apart; 2) one unprovoked (reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures occurring over the next 10 years; or 3) diagnosis of an epilepsy syndrome (Fisher et al. 2014).” Moreover, epilepsy is considered “resolved” for patients who had age-dependent epilepsy syndrome and are now past the applicable age, or those who have been seizure-free for the past ten years without anti-seizure drugs (ASDs) for the last five years.

1.2.1.1 Prevalence and Etiology

According to the Epilepsy Foundation, epilepsy is the fourth most common neurological disorder. Its prevalence has been reported to be range between 2.3-22.8 cases of epilepsy per 1,000 people in the general population worldwide (0.23-2.3%), and 6.8-8.5 cases in 1,000 of insured people in the United States alone (Bell, Neligan, and Sander 2014; H. Kim et al. 2016; Fiest et al. 2017;

Helmers et al. 2015). In 2017, ILAE commissioned a new classification system for seizure types and epilepsy types to improve the intuitiveness of the classification in addition to allowing for inclusion of previously unclassifiable seizure and epilepsy types (Figure 1.2.1-1) (Falco-Walter, Scheffer, and Fisher 2018). These will be discussed in more detail in the following section. Along each

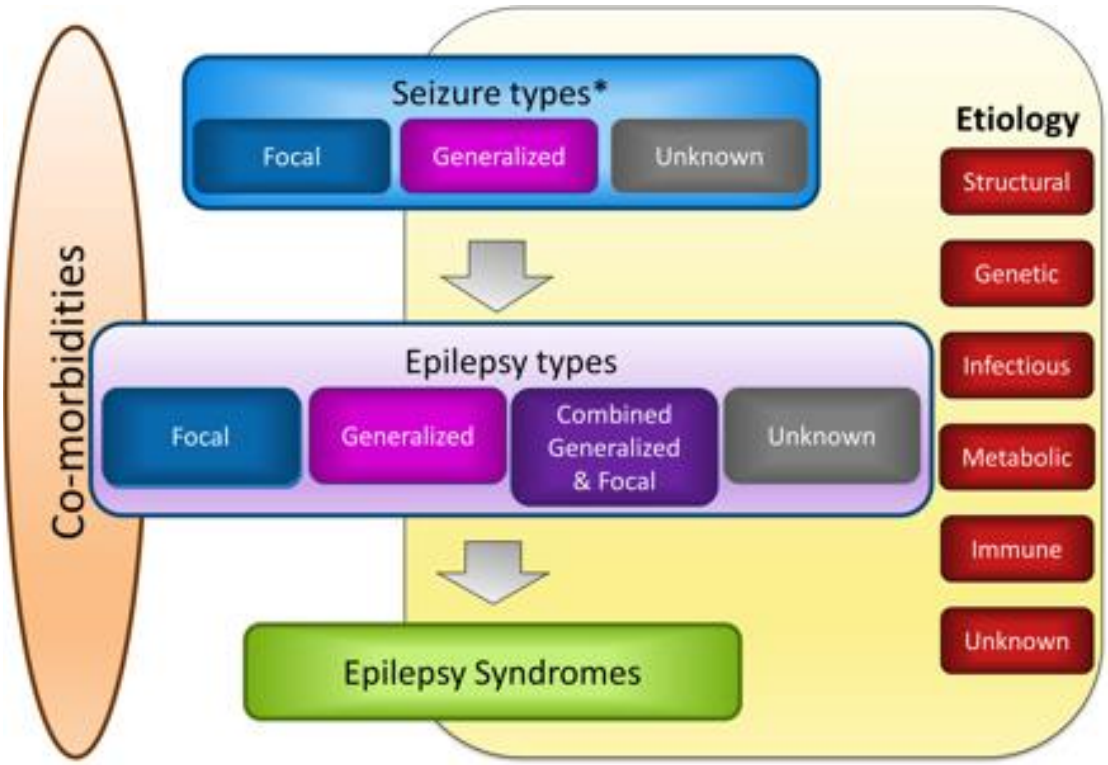

Figure 1.2.1-1 ILAE Framework for Classification of Epilepsies. Reproduced with persmission from Scheffer et al 2017.

step of the diagnostic pathway, the ILAE recommends that the clinician should attempt to identify the etiology of the patient's epilepsy (Scheffer et al. 2017). Within the new classification system, there are six non-hierarchical etiological categories with management implications, including: structural (i.e. neuroimaging finding inferred to cause the patient's seizures which may have resulted from a stroke, infection, trauma, genetic malformation, etc.), genetic (i.e. a known or presumed specific disease-causing gene variant believed to be pathogenic for

epilepsy), infectious (i.e. refers to a patient with seizures due to resolved infection), metabolic (e.g. uremia, pyridoxine-dependent seizures, cerebral folate deficiency), immune (i.e. when an autoimmune disease is the cause of new-onset epilepsy, like anti-NMDA receptor encephalitis), and unknown. A patient's epilepsy may be classified into more than one etiologic category, and the importance of each etiological group may depend on the patient's circumstance (e.g. a patient with tuberous sclerosis has a structural and genetic etiology, which would be critical for surgical and pharmacological considerations).

1.2.1.2 Seizure Semiology and Clinical Diagnosis

The starting point of the classification framework (Figure 1.2.1-1) is the operational identification of the seizure type, outlined in Figure 1.2.1-2 (Fisher et al. 2017). A seizure type is a grouping of seizure qualities for the purposes of communication in research, clinical care, and education. The framework is non-hierarchical, so that levels can be skipped or omitted with no other elaboration. However, use of additional classifiers are encouraged. Classification starts with the determination of the initial onset of the seizures (focal or generalized onset) and allows for classifications of seizures where the onset may be missed or obscured (unknown onset). If both motor and nonmotor seizures are present, the motor signs are usually overshadowing, unless the non-motor symptoms are obvious. Moreover, if a single seizure presents with a sequence of signs and/or symptoms, then the initial sign/symptom is used for the naming of the seizure. Finally, a seizure type of unknown onset can be classified at a later time with

additional clinical history and/or diagnostic tools. This may be more commonplace in settings where there is no access to EEG, video, or imaging technology.

The second step of the framework is epilepsy type. Diagnoses are made on clinical evaluation and is supported by EEG findings. Generalized epilepsy is

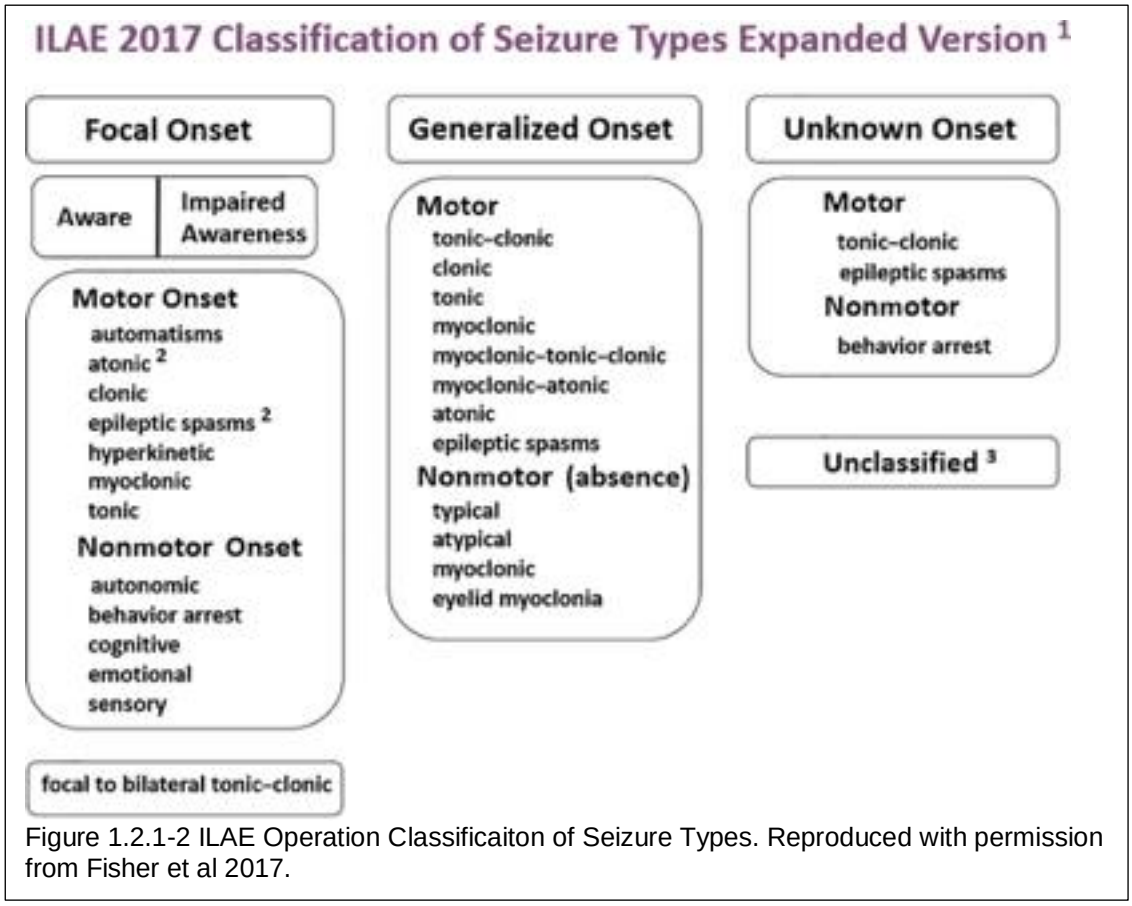

Figure 1.2.1-2 ILAE Operation Classificaiton of Seizure Types. Reproduced with permission from Fisher et al 2017.

typically diagnosed by the presence of interictal generalized spike-wave activity on EEG, while focal epilepsies can have unifocal/multifocal origins or seizures involving one hemisphere with interictal EEG showing focal epileptiform discharges. A new type of epilepsies, combined generalized and focal epilepsies, are for patients who have both epilepsy types (e.g. Dravet syndrome and Lennox-Gaustaut syndrome). Complementary to the updated epilepsies

categorization is an EEG diagnostic system composed by the ILAE Neurophysiology Task Force that can be applied to all epilepsy syndromes (Koutroumanidis et al. 2017). This system allows the clinician to determine the strength of EEG diagnosis and suggest further EEG tests where conclusive evidence is still lacking. Similar to seizure type classification, an unknown epilepsy type exists here if the clinician is unable to determine the type based on insufficient information (i.e. lack of EEG, or uninformative EEG).

Determination of an epilepsy syndrome is the last step of the framework. An epilepsy syndrome refers to a group of seizure types, age-dependent, EEG abnormalities, and imaging features that occur together. A syndrome may also have distinctive associated co-morbidities such as developmental impairment and/or psychiatric dysfunction. These features taken together may have associated prognostic and treatment implications.

1.2.1.3 Management of Epilepsy

As evidenced by the updated ILAE diagnosis criteria for epilepsy, estimating the recurrence risk following the first unprovoked seizure is essential not only to the diagnosis of epilepsy, but also for deciding whether treatment should be initiated. However, it should be noted that the decision to diagnose epilepsy is different and separate from the decision to treat. When considering whether treatment should be initiated, the clinician should be aware that the risk for a recurrent seizure is greatest within the first two years after the first seizure (21-45%), especially in the first year (Krumholz et al. 2015; Hauser et al. 1990; Annegers et

al. 1986; Hauser et al. 1982). The factors that have the highest level of evidence to be associated with an increased risk for seizure recurrence are having a prior brain insult and the presence of EEG epileptiform abnormalities (Krumholz et al. 2015). This risk for recurrent seizures appears to be lower for patients who are treated with antiseizure drug (ASD) therapy (Krumholz et al. 2015).

Antiseizure drugs are the mainstay of initial treatment for the majority of patients with epilepsy (Tables 1.2.1.1-4). Figure 1.2.1-3 depicts the different mechanisms of action of FDA-approved antiseizure drugs. In general, these mechanisms will result in decreased excitability of the postsynaptic neuron by decreasing excitatory input, increasing inhibitory input, or antagonizing voltage-gated cation channels.

Table 1.2.1-1 Drugs for the Management of Epilepsy (Part 1)

Antiseizure Drug	Antiseizure Mechanism(s) of Action	FDA-Approved Indications
Brivaracetam	Inhibition of synaptic vesicle protein 2A	Focal onset seizures, 4+ years
Cannabidiol	GPR55 antagonist and inhibition of VDAC1	Seizures associated with LGS or Dravet, 2+ years
Carbamazepine	Inhibition of voltage-gated sodium channels	Focal onset seizures with complex symptomatology, generalized onset tonic-clonic seizures, mixed seizure patterns. Not to be used for absence seizures.
Clobazam	$GABA_A$ receptor agonist	Adjunctive treatment of seizures associated with LGS, 2+ years
Clonazepam	$GABA_A$ receptor agonist	Absence seizures in those who failed succinimides, seizures associated with LGS, akinetic and myoclonic seizures
Eslicarbazepine	Inhibition of voltage-gated sodium channels	Focal onset seizures, 4+ years

GPR55: G protein-coupled receptor; VDAC1: adenosine reuptake channel; LGS: Lennox-Gastaut Syndrome; GABA: γ-aminobutyric acid.

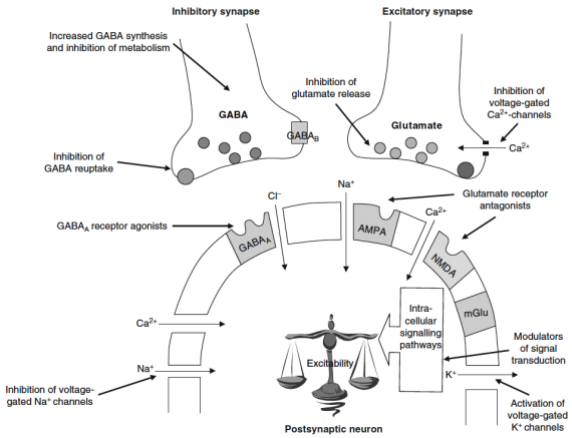

Figure 1.2.1-3 Mechanisms of Action of FDA-Approved Antiseizure Drugs. Reproduced with permission from C. Landmark 2008

Table 1.2.1-2 Drugs for the Management of Epilepsy (Part 2)

Antiseizure Drug	Antiseizure Mechanism(s) of Action	FDA-Approved Indications
Ethosuximide	Inhibition of T-type calcium channels	Absence seizures
Felbamate	NMDA receptor antagonist, inhibition of L-type calcium- and sodium-channels	Adjunctive treatment of focal- and generalized-onset seizures associated with LGS in children 2-14 years old; focal onset seizures with or without bilateral tonic-clonic. Not indicated for first-line.
Gabapentin	Inhibition of L-type calcium channels	Adjunctive treatment of focal onset seizures with or without bilateral tonic-clonic, 3+ years
Lacosamide	Enhance slow inactivation of sodium channels	Focal onset seizures, 4+ years
Lamotrigine	Inhibition of voltage-gated sodium channels	Adjunctive treatment of focal onset, primarily GTC, and generalized seizures of LGS in children 2+ years; focal onset seizures and seizures associated with LGS in adults
Levetiracetam	Inhibition of synaptic vesicle protein 2A	Adjunctive treatment of focal onset seizures, 1+ month; adjunctive treatment of myoclonic seizures associated with juvenile myoclonic epilepsy, 12+ years; adjunctive treatment of primary GTC seizures, 6+ years

NMDA: N-methyl-D-aspartate; LGS: Lennox-Gastaut Syndrome; GTC: generalized tonic-clonic

The selection of drug therapy will depend on patient-specific variables (e.g. gender, age, co-morbidities, co-medications, insurance coverage and/or financial situation) as well as ASD-specific variables (e.g. seizure type and/or epilepsy syndrome specific effectiveness, teratogenicity, pharmacokinetics, interaction potential, formulations, adverse effects) (Glauser et al. 2006). Non-pharmacological therapies for specific subpopulations of patients with epilepsy include a ketogenic diet (van der Louw et al. 2016; Nei et al. 2014), resective surgery (Kwon et al. 2016; West et al. 2015), and neurostimulation (i.e. vagal nerve stimulation, responsive neurostimulation) (Orosz et al. 2014; Hamilton et al. 2018; H. Chen et al. 2017; Skarpaas, Jarosiewicz, and Morrell 2019).

Table 1.2.1-3 Drugs for the Management of Epilepsy (Part 3)

Antiseizure Drug	Antiseizure Mechanism(s) of Action	FDA-Approved Indications
Oxcarbazepine	Inhibition of voltage-gated sodium- and N-type calcium channels	Focal onset seizures, 4+ years; adjunctive treatment of focal onset seizures, 2+ years
Perampanel	AMPA receptor antagonist	Focal onset seizures with or without bilateral tonic-clonic, 4+ years; adjunctive treatment of primary GTC seizures, 12+ years
Phenobarbital	$GABA_A$ receptor agonist	
Phenytoin	Inhibition of voltage-gated sodium channels	GTC and psychomotor seizures
Pregabalin	Inhibition of L-type calcium channels	Adjunctive treatment of focal onset seizures, 1+ month
Primidone	$GABA_A$ receptor agonist	GTC, psychomotor, and focal seizures

GTC: generalized tonic-clonic; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GABA: γ-aminobutyric acid

Table 1.2.1-4 Drugs for the Management of Epilepsy (Part 4)

Antiseizure Drug	Antiseizure Mechanism(s) of Action	FDA-Approved Indications
Rufinamide	Prolongs inactive state of voltage-gated sodium channels	Adjunctive treatment of seizures associated with LGS, 1+ year
Tiagabine	Inhibition of GAT-1	Adjunctive treatment of focal onset seizures, 12+ years
Topiramate	$GABA_A$ receptor agonist, AMPA/kainate receptor antagonist, inhibition of L-type calcium channels, inhibition of carbonic anhydrase (isozymes II and IV)	Focal onset or primary GTC seizures, 2+ years; adjunctive treatment for seizures associated with LGS, 2+ years
Vigabatrin	Irreversible inhibition of ABAT	Adjunctive treatment of refractory focal onset impaired awareness seizures, 10+ years; infantile spasms, 1 month-2 years
Valproic Acid	Inhibition of voltage-gated sodium channels and metabolism of GABA (via ABAT, ALDH5A1, and OGDH)	Focal onset impaired awareness seizures, absence seizures, and adjunctive treatment for patients with multiple seizure types that include absence, 10+ years
Zonisamide	Inhibition of T-type calcium channels, inhibition of carbonic anhydrase	Adjunctive treatment of focal onset seizures in adults

GTC: generalized tonic-clonic; GABA: γ-aminobutyric acid; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; LGS: Lennox-Gastaut Syndrome; GAT-1: GABA transporter 1; ABAT: GABA transaminase; ALDH5A1: succinate semialdehyde dehydrogenase; OGDH:

1.2.2 Seizure Emergencies: Status Epilepticus (SE)

1.2.2.1 Definition of Status Epilepticus: Differentiating from Acute Repetitive Seizures and Seizure Clusters

The ILAE recently commissioned an updated definition of status epilepticus (SE) that includes two operational dimensions indicating when treatment should be initiated and when long-term consequences may appear (Trinka et al. 2015). Status epilepticus is defined as “a condition resulting from either failure of

mechanisms responsible for seizure termination or from the initiation of mechanisms, which lead to abnormally prolonged seizures (after time point t_1). It is a condition, which can have long-term consequences (after time point t_2), including neuronal death, neuronal injury, and alternation of neuronal networks, depending on the type and duration of seizures." It is considered a life-threatening condition due to its risk for systemic complications and permanent brain injury. Tonic-clonic SE is defined as ≥5 minutes of tonic-clonic seizure activity, with a high risk for irreversible brain damage after 30 minutes of continued seizure activity. Both time points were determined from animal experiments and clinical research of convulsive SE, however, there is a lack of data for the other forms of SE. Focal SE with impaired consciousness is defined as ≥10 minutes of seizure activity, with a high risk for long-term consequences after at least 60 minutes. Finally, research is still ongoing and active to determine the time frame for prolonged absence seizure activity, and the time point at which long-term consequences is likely following absence SE.

Status epilepticus should be differentiated from another type of seizure emergency, called seizure clusters. Like SE, the failure of seizure terminating mechanisms appears to be the common pathophysiology in seizure clusters. However, unlike SE, there has not been a consensus on the definition of seizure clusters and is not listed in the ILAE Commission on Classification and Terminology (Fisher et al. 2017). Often also referred to as "acute repetitive seizures," "flurries," "cyclical, serial, repetitive, crescendo, and recurrent seizures," seizure clusters is generally defined as an acute series of seizures that

have short interictal periods with recovery of consciousness, have a recognizable onset, and whose pattern is different from the patient's usual seizure pattern (Dreifuss et al. 1998). Many clinical definitions are based on a seizure rate, for example, three or more seizures within 24 hours (Haut 2015). If left untreated, seizure clusters can progress into SE, increase emergency room visits, and is implicated as a risk for postictal psychosis (Haut 2015; Buelow et al. 2016; Jafarpour et al. 2019).

1.2.2.2 Prevalence and Etiology

In the United States alone, SE diagnosis accounted for 0.07% of over one billion hospitalizations recorded in the National Hospital Discharge Survey (NHDS) between 1979-2010 (Dham, Hunter, and Rincon 2014). Within this sample, the incidence increased from 3.5 to 12.5 per 100,000 person-years without a significant change in in-hospital mortality over the study period (9.2%). The incidence of SE has a bimodal distribution, with highest incidences in the first decade of life and after the fifth decade of life. The increase in estimated incidence has been attributed to more transparent and intuitive diagnostic criteria, increase in longer-living elderly population, and wider availability of EEG use in emergency departments (Betjemann et al. 2015; Leitinger et al. 2019). Similarly, a meta-analysis consisting of 47 international studies comprising of 80,307 SE cases also reported a crude annual incidence rate of 12.6 per 100,000 person-years (Lv et al. 2017). From these studies, stroke, nonadherence to antiseizure drug regimen, central nervous system infection, and trauma were

among the most significant causes of SE (Dham, Hunter, and Rincon 2014; Leitinger et al. 2019; Lv et al. 2017).

1.2.2.3 Status Epilepticus Subtypes and Clinical Diagnosis

Status epilepticus is classified by four axes: semiology, etiology, EEG correlates, and age (Trinka et al. 2015).

Axis 1: The semiology is the backbone of SE classification and refers to its clinical presentation, namely whether there are motor symptoms (i.e. convulsive versus nonconvulsive) and the degree of consciousness.

Axis 2: The etiology of SE is classified into whether the underlying cause is known. SE may result from known causes such as stroke, intoxication, trauma, brain tumor, or inappropriate ASD treatment.

Axis 3: Although there are no EEG criteria for SE and none of the ictal patterns is specific to a particular type of SE, EEG is still essential for the diagnosis of nonconvulsive SE. Specifically, the ILAE proposed the following terminology to describe EEG patterns in SE: 1) location (generalized, lateralized, bilateral independent, multifocal); 2) name of the pattern (periodic discharges, rhythmic delta activity or spike-and-wave/sharp-and-wave and subtypes); 3) morphology (sharpness, number of phases, polarity, absolute and relative amplitude); 4) time-related features (prevalence, frequency, duration, daily pattern, onset, dynamics; 5) modulation (stimulus-induced or spontaneous); and 6) effect of intervention on EEG.

Axis 4: Some forms of SE are seen more often in specific age groups (Table 1.2.2-1), some as a fundamental part of the electroclinical syndrome, while others occur when specific triggers are present.

Table 1.2.2-1 Status Epilepticus in Certain Electroclinical Syndromes. Reproduced with permission from Trinka et al 2015.

Table 5. SE in selected electroclinical syndromes according to age

SE occurring in neonatal and infantile-onset epilepsy syndromes
Tonic status (e.g., in Ohtahara syndrome or West syndrome)
Myoclonic status in Dravet syndrome
Focal status
Febrile SE
SE occurring mainly in childhood and adolescence
Autonomic SE in early-onset benign childhood occipital epilepsy (Panayiotopoulos syndrome)
NCSE in specific childhood epilepsy syndromes and etiologies (e.g., Ring chromosome 20 and other karyotype abnormalities, Angelman syndrome, epilepsy with myoclonic-atonic seizures, other childhood myoclonic encephalopathies; see Appendices 1–3)
Tonic status in Lennox-Gastaut syndrome
Myoclonic status in progressive myoclonus epilepsies
Electrical status epilepticus in slow wave sleep (ESES)
Aphasic status in Landau-Kleffner syndrome
SE occurring mainly in adolescence and adulthood
Myoclonic status in juvenile myoclonic epilepsy
Absence status in juvenile absence epilepsy
Myoclonic status in Down syndrome
SE occurring mainly in the elderly
Myoclonic status in Alzheimer's disease
Nonconvulsive status epilepticus in Creutzfeldt-Jakob disease
De novo (or relapsing) absence status of later life

These forms of SE may be encountered prevalently in some age groups, but not exclusively.

In addition to basing SE subtypes on clinical presentation of the condition, SE can also be characterized by its responsiveness to drug therapy. Once seizure activity is considered prolonged, the patient is considered to have “early SE.” If the seizures still persist after an adequate dose of a benzodiazepine (first-line drug therapy), the patient would have “established SE.” Similarly, patients failing second-line drug therapies have “refractory SE.” Finally, if patients are

unable to be weaned off of their anesthetizing third-line agent and/or have breakthrough seizures while on third-line agent(s), they have “super-refractory SE.”

1.2.2.4 Pathophysiology of Status Epilepticus and Mechanisms of Drug Resistance

It has become increasingly recognized by the scientific community that SE results from the failure of a seizure to hypothetically cross the transition from an ictal to post-ictal state (Figure 1.2.2-1) (Walker 2018). Walker proposed that

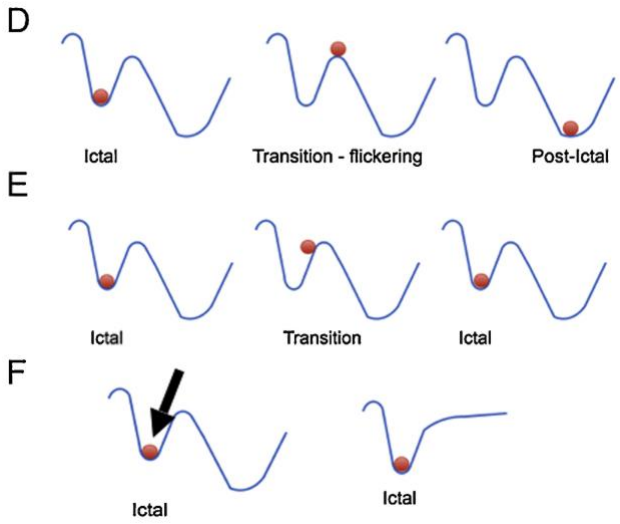

Figure 1.2.2-1 Ictal, Transition, and Post-Ictal States. Ictal, Transition, and Post-Ictal States. D) Example of a seizure that successfully transitions into the post-ictal state; E) example of a seizure that is able to reach but not cross the transition into the post-ictal state; F) example of a seizure that remains in the ictal state due to the mechanisms of seizure promotion or where there is no post-ictal state due to SE etiology or neuronal network damage. Reproduced with permission from Walker M 2018.

seizure termination is dependent on the presence of ictal and post-ictal states, and that the critical transition must occur in order to reach the post-ictal state. By encouraging this transition, ASDs can facilitate moving the brain state towards the post-ictal state. In addition, increased seizure duration has been shown to

increase the chance of self-sustained seizure activity in animal models of SE and patients with SE (Mazarati, Wasterlain, et al. 1998; Shinnar et al. 2008; Delorenzo et al. 1999).

The self-sustaining nature of SE is reminiscent of long-term potentiation (LTP), the phenomenon behind memory and learning. LTP is a process characterized by the strengthening of synaptic connections between neurons following frequent stimulation (Purves et al. 2001). Following a strong depolarization of the postsynaptic neuron and with continued stimulation, there is increased surface expression of postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors allowing for a stronger connection between the two neurons. In fact, the perforant path stimulation model of SE is followed by increased LTP in the perforant pathway (Mazarati, Wasterlain, et al. 1998; Reddy and Kuruba 2013).

Pathophysiological changes on the cellular and molecular level promote continued seizure activity and pharmacoresistance. Following prolonged seizure activity, N-methyl-D-aspartate (NMDA) receptors increase in surface expression (Naylor et al. 2013), presynaptic adenosine A1 receptor, neuronal potassium-chloride cotransporter (KCC2) and $GABA_B$ receptor activities become downregulated (Avsar and Empson 2004; Hamil, Cock, and Walker 2012; Silayeva et al. 2015; Kaila et al. 2014; Chandler et al. 2003; Leung 2019), and AMPA receptors lose their GluA2 subunit (Rajasekaran, Todorovic, and Kapur 2012; Malkin et al. 2016). These AMPA receptors then become permeable to calcium, amplifying the accumulation of intracellular calcium and increasing the

risk for neuronal death (Cull-Candy, Kelly, and Farrant 2006). Taken together, these observations support the concept that continued seizure activity can

Table 1.2.2-2 Factors that Promote/Diminish Self-Sustaining Seizure Activity. Reproduced with permission from Niquet et al 2016.

Table 1. Initiators and blockers of self-sustaining status epilepticus

Initiators	Blockers of initiation phase	Blockers of maintenance phase
• Low Na^+, high K^+	• Na^+ channel blockers	• NMDA antagonists
• $GABA_A$ antagonists	• $GABA_A$ agonists	• Tachykinin antagonists
• Glutamate agonists: NMDA, AMPA, kainate, low Mg^{2+}, low Ca^{2+}, stimulation of glutamatergic pathways	• NMDA antagonists, high Mg^{2+}	• Galanin
	• AMPA/kainate antagonists	• Dynorphin
	• Cholinergic muscarinic antagonists	
• Cholinergic muscarinic agonists, stimulation of muscarinic pathways	• SP, neurokinin B antagonists	
• Tachykinins (SP, NKB)	• Galanin	
• Galanin antagonists	• Somatostatin	
• Opiate δ agonists	• NPY	
• Opiate κ antagonists	• Opiate δ antagonists	
	• Dynorphin (κ agonist)	

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NKB, neurokinin B; SP, substance P; NPY, neuropeptide Y.

strengthen seizure-promoting and/or deplete seizure-terminating mechanisms (Table 1.2.2-2).

When seizures become self-sustaining, resistance to drugs, particularly benzodiazepines, develops progressively over time (Kapur and Macdonald 1997). Synaptic γ-aminobutyric acid $(GABA)_A$ receptors (those containing a δ-subunit) internalize after one hour of lithium/pilocarpine-induced SE *in vivo* (Naylor, Liu, and Wasterlain 2005). This phenomenon explains why benzodiazepines are highly effective within the first five minutes of seizure activity, but not effective after 45 minutes (Kapur and Macdonald 1997). This process is initiated by the activation of NMDA receptors and consequently the calcium-dependent internalization of synaptic $GABA_A$ receptors (Rice and Delorenzo 1999; Niquet et al. 2016). As shown *in vivo*, the decrease in inhibitory post-synaptic potentials from the loss of synaptic $GABA_A$ receptors causes a loss

in inhibitory tone of hippocampal circuits and promotes a pro-seizure state (Naylor, Liu, and Wasterlain 2005).

1.2.2.5 Management and Prognosis of Convulsive Status Epilepticus

While the optimal therapy for convulsive SE is still uncertain (i.e. there is no intervention that is successful in 100% of cases), there are established guidelines for its management. In 2016, the American Epilepsy Society and Epilepsy Foundation published a treatment algorithm consisting of the best current medical management of convulsive SE based off of clinical trial evidence (Glauser et al. 2016). In total, 38 relevant published randomized, controlled trials and four meta-analyses were identified, and pharmaceutical companies provided information on three randomized, controlled trials. The following is a summary of the consensus guidelines (Figure 1.2.2-2), whereas safety and effectiveness of specific therapies are discussed in the next section.

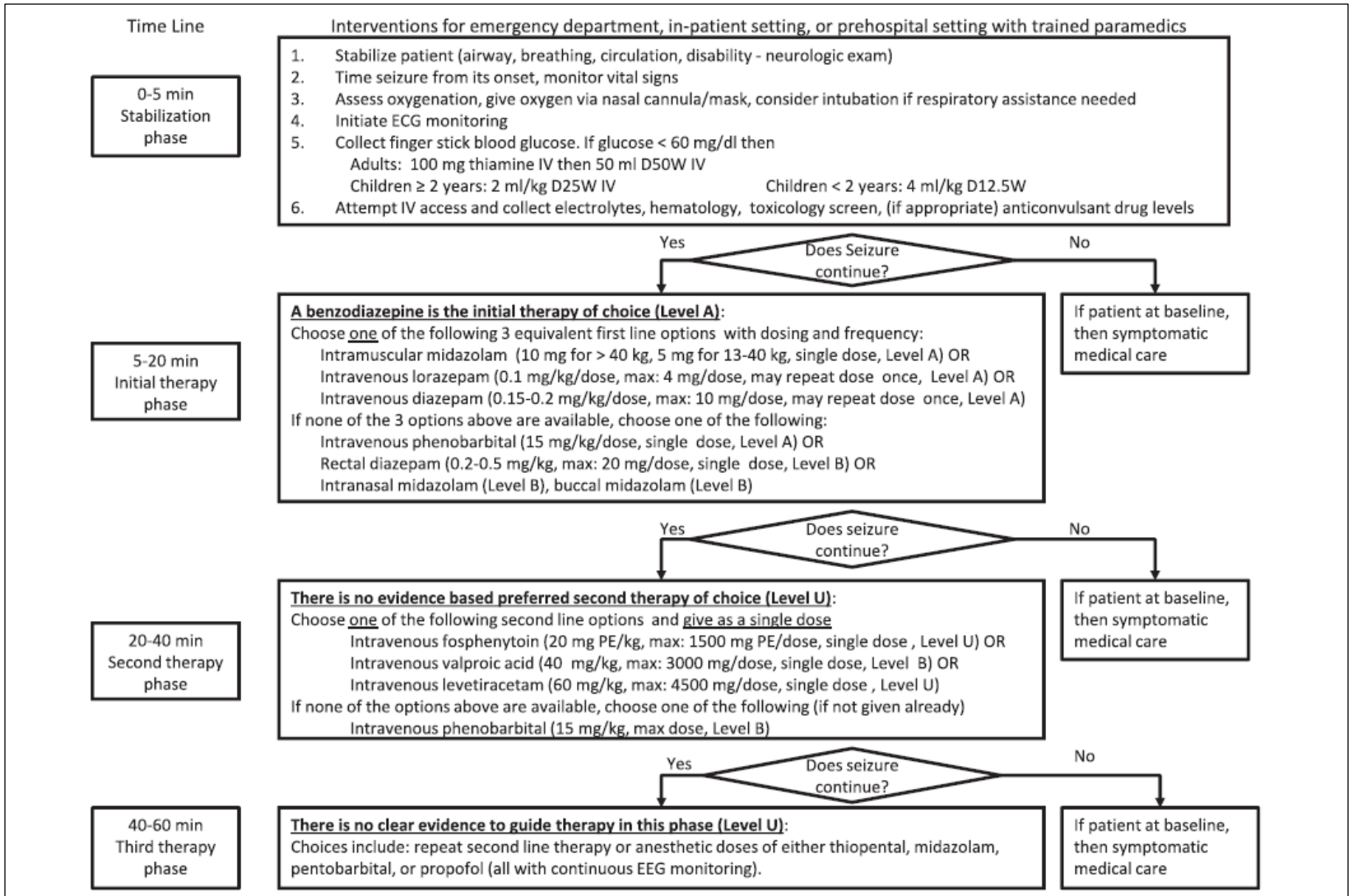

Figure 1.2.2-2 Algorithm for Management of Convulsive Status Epilepticus. Reproduced with permission from Glauser et al 2016.

1.2.2.5.1 Early SE

Within the first five minutes of a convulsive seizure, patients should be stabilized (airway, breathing, circulation), blood glucose should be evaluated, intravenous (IV) access should be attempted for collection of serum electrolytes, complete blood count, toxicology screen, ASD level (if applicable), and administration of drugs. The goal of these early assessments is to rectify any reversible causes for seizure activity (e.g. hypoglycemia, drug withdrawal, electrolyte disturbance).

After five minutes of convulsive seizure activity, a benzodiazepine should be given either intravenously (lorazepam 0.1 mg/kg/dose or diazepam 0.15-0.2 mg/kg/dose, may repeat once) or intramuscularly (IM; midazolam 10 mg if >40 kg, given once). The goal of drug therapy is rapid termination of seizures and prevention of recurrent seizure activity. Benzodiazepines (BZDs) have demonstrated their safety, efficacy, and tolerability as the first-line therapy for SE with a high level of evidence from four RCTs (Treiman et al. 1998; Alldredge et al. 2001; Silbergleit et al. 2012; Chamberlain et al. 2014). Looking across these studies, 43-74% of cases successfully terminated within 20 minutes of benzodiazepine administration, 11-39% of these cases had seizure recurrence within the study period, 29-57% of all cases required intensive care unit admissions, and up to 27% of all cases resulted in death.

Most of the clinical trials were conducted using the IV route of administration, requiring a trained technician to establish IV access. Ideally, SE treatment would be administered immediately following the start of seizure

activity and likely in the pre-hospital setting by a parent or caregiver. The Rapid Anticonvulsant Medication Prior to Arrival Trial (RAMPART) demonstrated that rapid administration of treatment impacts outcome (Silbergleit et al. 2012). RAMPART compared the efficacy of intramuscular midazolam (IM MDZ) to intravenous lorazepam (IV LZP) in stopping SE prior to emergency department arrival without requiring rescue therapy, and showed that although the time until seizure termination was similar in both treatment groups, the time saved by using the IM route significantly affected its efficacy positively.

A disadvantage of BZD use is that common side effects include impaired cognition, psychomotor slowing and sleepiness that can last into the next day, decreasing the patient's ability to return to school or work (Roehrs et al. 1986; Kay et al. 2016; Griffin et al. 2013). Furthermore, BZDs like diazepam and midazolam are metabolized by the cytochrome P450 isozyme 3A4 (CYP3A4), and are vulnerable to drug-drug interactions with common ASDs like phenytoin, phenobarbital, carbamazepine (Griffin et al. 2013; Indiana University Department of Medicine Clinical Pharmacology 2019). In addition, as seizure duration increases, synaptic $GABA_A$ receptors become increasingly internalized, and BZDs lose their efficacy (Wasterlain and Chen 2008). Therefore, although effective in most cases, the first-line management of SE could be improved. The development of an alternative therapy that can be administered either intravenously or intramuscularly has the potential to improve outcomes in patients with SE.

1.2.2.5.2 Established SE (ESE)

Second-line therapies include IV fosphenytoin (20 mg phenytoin-equivalent /kg, single dose), valproic acid (40 mg/kg, single dose), or levetiracetam (60 mg/kg, single dose). The goal of drug therapy is rapid termination of seizures and prevention of recurrent seizures. There is a moderate level of evidence demonstrating lack of significant difference in efficacy between these three therapies (Malamiri et al. 2012; Agarwal et al. 2007; W. B. Chen et al. 2011; U. Misra, Kalita, and Maurya 2012; Lyttle et al. 2019; Dalziel et al. 2019; Gujjar et al. 2017; Mundlamuri et al. 2015; Nene et al. 2019). Across these studies, 50-88% of cases successfully terminated without seizure recurrence within at least six hours following study drug administration, 20-73% of these cases had seizure recurrence within 24 hours, 23-64% of all cases required intensive care unit admissions, and up to 43% of cases resulted in death. There is large variability in response depending primary outcomes of interest, and possibly in the open-label nature of the studies.

The use of these second-line therapies do not come without risks. Systemic complications such as systemic hypotension, Stevens-Johnson Syndrome, hyperammonemia, and hematologic abnormalities (e.g. thrombocytopenia, pancytopenia, agranulocytosis) have been reported in clinical trials and post-marketing settings for these ASDs (KEPPRA® [package insert] 2017; Depacon [package insert] 2019; CEREBYX® [package insert] 2019). These observations emphasize the need for more effective therapies to prevent

the large proportion of intensive care unit admissions following failure of second-line therapies.

1.2.2.5.3 Refractory SE (RSE)

There is currently insufficient evidence to guide therapy due to the rarity of the condition and difficulty in the interpretation of findings due to the complex interaction of drugs used in parallel and co-morbidities at this later stage of SE. Third-line therapies provided in the guidelines include repeating second-line therapy or anesthetic medications including IV midazolam, thiopental, pentobarbital, or propofol. Due to the ethical challenges in randomization of interventions in intensive care settings, there is a lack of prospective, randomized, blinded and controlled studies in refractory SE. Instead, numerous small, prospective, open-label studies that compare the safety and effectiveness of ketamine, continuous infusion of midazolam or diazepam, propofol, and barbiturates are available (Rosati et al. 2012; Rossetti et al. 2011; Morrison et al. 2006; Mehta, Singhi, and Singhi 2007; Koul et al. 2002; Ulvi et al. 2002). Without the rigorous controlled trials, registries and audits could also provide useful information on general consensus of the management of refractory SE with some limitations. Early results of a multinational, prospective audit of 488 patients with refractory and super-refractory SE reported that the most widely used anesthetic as first-choice is midazolam, followed by propofol and barbiturates (Ferlisi et al. 2015). From this survey, 74% of cases recovered from RSE, 22% died, and 4% had treatment withdrawn due to futility. Although anesthetic agents are useful in suppressing seizures, they are associated with a higher risk of systemic

complications death independent of underlying medical conditions (Sutter et al. 2014). There is an unmet need for better control of refractory SE, ideally before the need for burst-suppression.

1.2.2.5.4 Super-refractory SE (SRSE)

There remains no standard of care for the treatment of SRSE for reasons similar to refractory SE. Interventions that have been evaluated at this stage of SE include perampanel (Beretta et al. 2018; Rohracher et al. 2015; Brigo et al. 2018), allopregnanolone (Broomall et al. 2014; Rosenthal et al. 2017), ketamine (Höfler et al. 2016), stiripentol (A. Strzelczyk et al. 2015; Uchida et al. 2018), rufinamide (Thompson and Cock 2016), cannabidiol oil (Rosemergy, Adler, and Psirides 2016), inhaled anesthetics, barbiturates, electroconvulsive therapy (Pinchotti, Abbott, and Quinn 2018; Chan et al. 2018), thalamic deep brain stimulation (Lehtimäki et al. 2017), and ketogenic diet (Farias-Moeller et al. 2017; Appavu et al. 2016; Thakur et al. 2014).

1.3 Using Canine Status Epilepticus as a Model of Human Status Epilepticus

1.3.1 Canine Epilepsy

1.3.1.1 Prevalence and Etiology

Canine epilepsy is practically defined as having at least two unprovoked epileptic seizures greater than 24 hours apart (Mette Berendt et al. 2015). In veterinary practice, dogs with epilepsy are among the most common neurological diagnosis. The true prevalence of epilepsy in dogs is unknown but has been estimated to range between 0.55-5.7% in the general dog population (Loscher et al. 1985;

Heske et al. 2014; Kearsley-Fleet et al. 2013; Michael Podell, Fenner, and Powers 1995). The etiology of canine epilepsy as varied as that of human epilepsy. Following the classification and terminology system published by the ILAE for human epilepsy, the International Veterinary Epilepsy Task Force (IVETF) has adopted proposals for the canine epilepsy classification and terminology system that reflect the evolving understanding of the human disease. Epilepsy classified by etiology are divided into two categories: idiopathic (purely genetic, a combination of genetic and epigenetic influences, or unknown cause and no indication of structural epilepsy), and structural (identified cerebral pathology) (Mette Berendt et al. 2015). In contrast, human epilepsy etiology is broken into six categories (i.e. structural, genetic, infectious, metabolic, immune, and unknown) and more than one category can be used to describe a patient's epilepsy (Scheffer et al. 2017). Some breeds with suggested inherited idiopathic epilepsies include Beagles, Boxers, Border Collies, German Shepherds, Labrador Retrievers, and Vizlas (Monteiro et al. 2012; Ekenstedt, Patterson, and Mickelson 2012; Bielfelt, Redman, and McClellan 1971; Nielen, Janss, and Knol 2001; Jaggy et al. 1998; Patterson et al. 2003).

1.3.1.2 Seizure Semiology and Clinical Diagnosis

Epileptic seizures are classified as either focal (clinical signs indicating activity starts in a localized area in the brain), generalized (clinical signs indicating activity starts in both cerebral hemispheres from the start), and focal epileptic seizure evolving to become generalized (clinical signs indicating activity starts in

a localized area in the brain and spreads to involve both cerebral hemispheres) (Mette Berendt et al. 2015). Focal epileptic seizures can present as motor (e.g. facial twitches, repeated rhythmic jerks of one extremity, or rhythmic blinking), autonomic (e.g. dilated pupils, hypersalivation, vomiting), or behavioral (e.g. episodic change in behavior such as anxiousness, unexplainable fear reactions, or abnormal attention seeking). Generalized epileptic seizures most often present as tonic, clonic or tonic-clonic epileptic seizures in dogs sometimes with expulsion of urine or feces. Non-convulsive generalized epileptic seizures (atonic) in dogs, called ‘drop attacks’, are caused by the sudden loss of muscle tone. Finally, the most common seizure type observed in dogs is focal epileptic seizures evolving into generalized epileptic seizures. The focal epileptic seizure is brief (seconds to minutes) and is followed by a convulsive stage with bilateral tonic, clonic, or tonic-clonic activity.

Diagnosis of epileptic seizures includes two steps: establish whether events animal are demonstrating are truly representative of epileptic seizures, and identifying the cause of the epilepticus seizure (De Risio et al. 2015). The first step is particularly difficult without observation of characteristic electroencephalographic (EEG) changes and physical manifestation of seizures. However, this is not practical in veterinary medicine and there is no standard protocol for acquiring EEG in dogs. Therefore, the current practice is to obtain a detailed and accurate history of events from pet owners, and completion of a standardized epilepsy questionnaire with video recording when available. The veterinarian must be able to distinguish epileptic seizures from other non-

epileptic episodic paroxysmal events (e.g. syncope, narcolepsy, idiopathic head tremor).

After the diagnosis of epileptic seizures, the next step is the determination of their cause which will have implications on the treatment and prognosis. Reactive seizures can result from intoxications (e.g. organophosphates, ethylene glycol) or from systemic metabolic disorders (e.g. electrolyte imbalance, hypoglycemia, hypothyroidism). Structural disorders resulting from infectious, inflammatory, traumatic, or neoplastic disease can result in epileptic seizures. Neurological examination if often abnormal and may present as asymmetric neurological deficits in dogs. Magnetic resonance imaging (MRI) of the brain and cerebrospinal fluid (CSF) analysis is recommended to rule out structural epilepsy. After exclusion of reactive seizures, MRI is CSF analysis is recommended in dogs with age of seizure onset <6 months or >6 years, status epilepticus or cluster seizure, interictal neurological abnormalities, or a previous presumptive diagnosis of idiopathic epilepsy and drug resistance with a single antiseizure drug titrated to the highest tolerable dose. The criteria for the diagnosis of idiopathic epilepsy is three-tiered: 1) a history of two or more unprovoked epileptic seizures occurring at least 24 hours apart, the age of seizure onset between 6 months and 6 years of age, unremarkable interictal physical and neurological examination, and no clinically significant abnormalities on blood tests and urinalysis; 2) unremarkable fasting and post-prandial bile acids, brain MRI, and CSF analysis; and 3) identification of ictal or interictal EEG

abnormalities characteristic for seizure disorders (criteria derived from human medicine).

1.3.1.3 Management of Canine Epilepsy

Antiseizure drugs (ASD) are the mainstay of therapy for idiopathic epilepsy (Bhatti et al. 2015; M. Podell et al. 2016; Marios Charalambous, Brodbelt, and Volk 2014) (Table 1.3.1-1). In contrast to the goal of ASD therapy in humans of seizure freedom, the goal of therapy in dogs is decrease seizure frequency, duration, or severity with limited/acceptable side effects to maximize the dog's and owner's quality of life. When the decision has been made to initiate ASD therapy, the selection of ASD is made by a veterinarian's recommendation and depends on the dog (i.e. the seizure type, frequency, etiology), the drug (i.e. side effect profile, drug interactions, frequency of administration), and the owner (i.e. financial situation, lifestyle).

Aside from ASD therapy, there are nonpharmacological interventions for the management of canine epilepsy. These include vagal nerve stimulation, medium chain triglyceride (MCT)-based diet, and acupuncture (Munana et al. 2002; Hong Law et al. 2015; Goiz-Marquez et al. 2009; Klide, Farnbach, and Gallagher 1987).

Table 1.3.1-1 Antiseizure Drugs Used in the Management of Canine Epilepsy

Antiseizure Drug	Antiseizure Mechanism(s) of Action	Place in Therapy
Phenobarbital	$GABA_A$ receptor agonist	First-line; high recommendation for monotherapy and moderate recommendation for adjunctive therapy
Imepitoin	Partial $GABA_A$ receptor agonist	First-line; high recommendation for monotherapy and low recommendation for adjunctive therapy
Bromide	Hyperpolarization of neuron via bromide influx	Adjunctive to PB or monotherapy if hepatoxicity occurs with PB; moderate recommendation for monotherapy and adjunctive therapy
Primidone	$GABA_A$ receptor agonist (phenobarbital pro-drug)	No advantage to using primidone over phenobarbital; not recommended for monotherapy or adjunctive therapy
Felbamate	NMDA receptor antagonist, inhibition of L-type calcium- and sodium-channels	Adjunctive to PB; insufficient evidence to recommend its use
Gabapentin	Inhibition of L-type calcium channels	Adjunctive to PB; insufficient evidence to recommend its use
Pregabalin	Inhibition of L-type calcium channels	Adjunctive to PB; insufficient evidence to recommend its use
Levetiracetam	Inhibition of synaptic vesicle protein 2A	Adjunctive to PB; low recommendation for monotherapy and moderate recommendation for adjunctive therapy
Topiramate	$GABA_A$ receptor agonist, AMPA/kainate receptor antagonist, inhibition of L-type calcium channels, inhibition of carbonic anhydrase (isozymes II and IV)	Adjunctive to PB; insufficient evidence to recommend its use
Zonisamide	Inhibition of T-type calcium channels, inhibition of carbonic anhydrase	Adjunctive to PB; low recommendation for monotherapy and moderate recommendation for adjunctive therapy

GABA: γ-aminobutyric acid; NMDA: N-methyl-D-aspartate; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

1.3.2 Canine Status Epilepticus

1.3.2.1 Prevalence and Etiology

As in humans, SE in dogs is defined as continuous seizure activity lasting for at least five minutes or as two or more discrete seizures without complete recovery of consciousness in between (Blades Golubovic and Rossmeisl 2017a). Epidemiologic studies on SE in dogs report a prevalence ranging 2.5-59% in dogs admitted into a teaching hospital for seizures (Zimmermann et al. 2009; Saito et al. 2001; Bateman and Parent 1999) and 0.44-0.7% in the all dogs admitted into a teaching hospital (Zimmermann et al. 2009; Bateman and Parent 1999). Although a rare condition in the general dog population, SE occurs more often in dogs without idiopathic epilepsy. A retrospective case-control study done in 50 dogs that exhibited generalized convulsive tonic-clonic (GCTC) SE compared with 50 dogs that exhibited non-SE GCTC seizures found that dogs in the non-SE group were more than twice as likely to have idiopathic epilepsy than symptomatic/reactive epileptic seizures (Platt and Haag 2002). Similarly, Zimmerman et al found that dogs with reactive seizures had a 1.87 relative risk of developing SE compared to all other dogs (Zimmermann et al. 2009).

1.3.2.2 Canine Status Epilepticus Subtypes and Clinical Diagnosis

Similar to human SE, canine SE is classified into convulsive and nonconvulsive SE. However, contrary to human SE, canine SE does not yet have subclassifications of early, established, refractory, and super-refractory SE. The

majority of animals that develop SE will present with a phenotype consistent with convulsive GCTC SE. Although it has been recognized by veterinary medicine, nonconvulsive SE has not been frequently documented, likely due to the underutilization of EEG in the diagnosis of epileptic seizures. A retrospective case series recently reported that 20% and 12% of 104 dogs and cats with any type of EEG procedure performed had electrographic seizures or electrographic SE, and were associated with a 48% and 50% in-hospital mortality rates, respectively (Granum et al. 2019). In addition, 81% of these animal patients with electrographic seizures had no or only subtle signs of seizure activity, emphasizing the need for future standardizing EEG procedures to detect electrographic seizure activity.

The diagnostic algorithm for SE for dogs consists of physical diagnostics (i.e. physical exam, neurological exam), point of care tests (e.g. blood glucose, electrolytes, electrocardiogram, complete blood count), and patient-specific diagnostics (e.g. evidence of toxic or metabolic encephalopathy, brain MRI, CSF analysis, serum ASD concentration) (Blades Golubovic and Rossmeisl 2017a). Pet owners should be questioned about the patient's history of seizures or neurological disease, history of metabolic disease, potential exposure to toxins, and possibility of trauma.

1.3.2.3 Management and Prognosis of Canine Status Epilepticus

The goals of treatment of canine SE is similar to that of the treatment of human SE and include seizure termination and prevention of further seizures while managing systemic complications of SE and, if possible, the underlying cause (Glauser et al. 2016; Blades Golubovic and Rossmeisl 2017b). The first step of treating SE is stabilization of the canine patient by assess airway, breathing, and

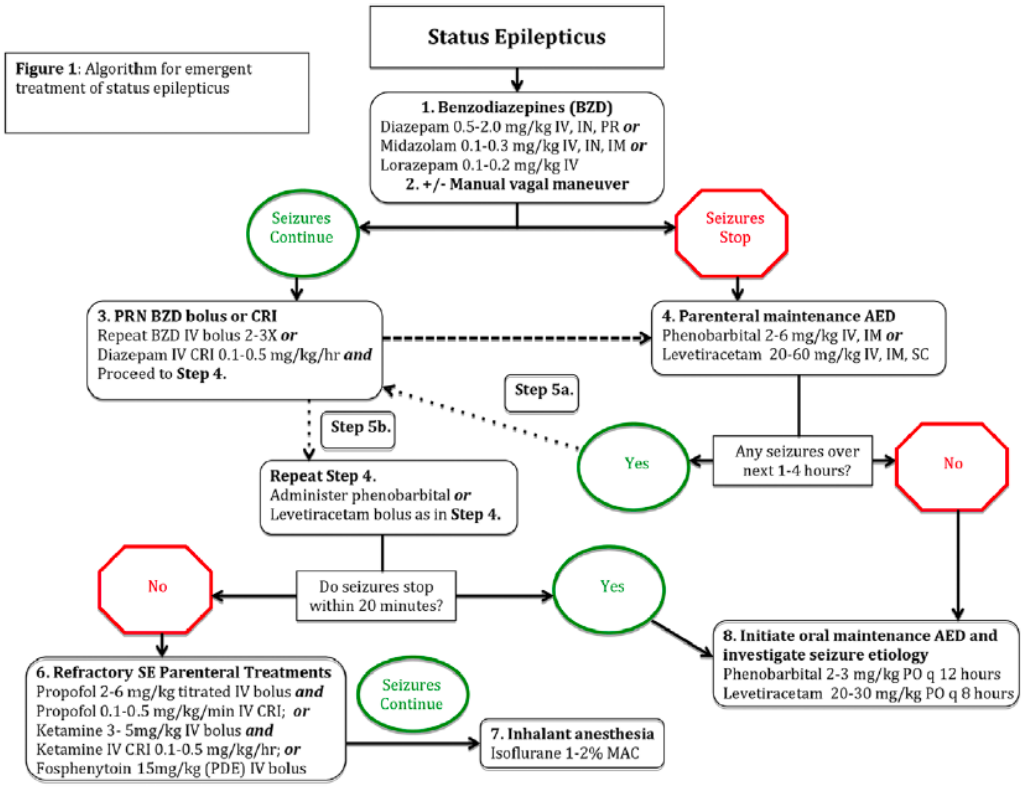

Figure 1.3.2-1 Management of Status Epilepticus in Dogs and Cats
Reproduced with permission from Blades Golubovic and Rossmeisl 2017b

circulation (Figure 1.3.2.3-1). An intravenous line should be established for pretreatment blood sampling, drug delivery, and intravenous fluids. Blood glucose, electrolytes, complete blood count, toxicology screen (if exposure is known or suspected), and ASD concentrations should be measured at this time.

The recommended first-line agent is a benzodiazepine (i.e. diazepam 0.5-2 mg/kg IV/IN/PR, lorazepam 0.1-0.2 mg/kg IV, or midazolam 0.1-0.3 mg/kg 36

IV/IN/IM) with or without a manual vagal maneuver. Benzodiazepines rapidly distribute into the central nervous system (CNS) and redistributes into fat and muscle quickly. Therefore, repeat dosing can cause accumulation in the CNS and unexpected CNS depression. For this reason, after two to three doses, a constant rate infusion (CRI) of another ASD should be initiated. Of note, diazepam and lorazepam are solubilized with the addition of propylene glycol. With rapid IV administration, propylene glycol can cause hypotension and phlebitis. Alternatively, instead of a second or third dose of a BZD bolus, a diazepam CRI 0.1-0.5 mg/kg/hr can be administered followed by second-line parenteral dosing of LEV 30-60 mg/kg IV/SC or PB 2-6 mg/kg IV (Patterson 2014b).

If seizures terminate after the initial BZD dose, the canine patient will receive second-line parenteral dosing of a maintenance ASD (potentially more than once) before initiation of oral maintenance ASD (PB 2-3 mg/kg by mouth every 12 hours or LEV 20-30 mg/kg by mouth every 8 hours). However, if seizures fail to terminate within 20 minutes after several doses of BZD and parenteral maintenance ASD bolus, third-line parenteral treatments used for refractory SE (propofol 2-6 mg/kg IV bolus + 0.1-0.5 mg/kg/min IV CRI, ketamine 3-5 mg/kg IV bolus + 0.1-0.5 mg/kg/hr CRI, or fosphenytoin 15 mg/kg [phenytoin equivalent] IV bolus). Finally, if seizures continue after third-line agents, isoflurane 1-2% minimum alveolar concentration should be used to induce seizure suppression.

The mortality rates of canine patients with SE have been reported to range between 25-45%, with death occurring in 2.1-8% and mostly from euthanasia (Zimmermann et al. 2009; Bateman and Parent 1999; Saito et al. 2001). Dogs with structural epilepsy were found to have lower survival probably following SE than dogs with idiopathic epilepsy (HR 0.11 [95% CI 0.03;0.37]) and dogs with reactive seizures (HR 0.16 [95% CI 0.05;0.58]) (Zimmermann et al. 2009).

1.3.2.4 Canine Status Epilepticus Clinical Trials

In general, there is a lack of extensive published clinical trial evidence for canine SE. Much of the treatment recommendations are based on human clinical trial experience. Dosing for ASDs recommended in the treatment of canine SE largely stems from targeting the serum drug levels within the range considered therapeutic for people. To date, there are a handful of case series and controlled clinical trials for the treatment of early and refractory canine SE.

Aside from one published case series on the use of rectal diazepam (PR DZP) for canine cluster seizures, there is one open-labeled, randomized, parallel group clinical trial comparing the effectiveness of an atomized intranasal midazolam (IN MDZ) to PR DZP for the first-line management of canine SE in client-owned animals (Michael Podell 1995; M. Charalambous et al. 2017). Success was defined as seizure termination within 5 minutes without recurrence of seizures within 10 minutes. IN MDZ (n=20) and PR DZP (n=15) terminated SE in 70- and 20-% of cases, respectively, and all dogs showed sedation and ataxia. Median time to seizure cessation was 47 seconds (range, 6-280) in the IN MDZ

group, whereas it was 214 seconds (range, 204-290) in the PR DZP group. Twenty-one percent of successful IN MDZ cases did not have a relapse of seizures, while all of the successful PR DZP cases relapsed (median time to relapse was 904 and 645 seconds in the IN MDZ and PR DZP groups, respectively). IN MDZ was more effective in terminating SE, with a higher rate of seizure termination, faster onset of action, and lower rate of seizure relapse.

For refractory canine SE, one case series and two single case reports described success using rectal LEV, IV ketamine, and controlled hypothermia, respectively (Cagnotti et al. 2018; Serrano, Hughes, and Chandler 2006; Hayes 2009). Two randomized, double-masked, placebo-controlled trials evaluated the clinical efficacy of IV LEV and IV fosphenytoin (FOS) in addition to IV DZP for the treatment of canine SE or acute repetitive seizures (Hardy et al. 2012; Patterson et al. 2015). In the IV LEV study, a responder was defined as a dog that had no additional seizures after study drug administration for the following 24 hours. Of the 19 cases (n=9 IV LEV, n=10 placebo), 56% in the IV LEV group responded to treatment compared to 10% in the placebo group (p=0.06). There was no difference in the mortality rates in either group (p=0.6), and no dogs required pentobarbital or propofol treatment. IV LEV was safe and potentially effective for the treatment of SE in addition to IV DZP. In contrast, a responder in the IV FOS study was defined as a dog that no additional seizures after drug administration for the following 2- and 12-hours. Of the 32 dogs (n=22 IV FOS, n=9 IV placebo), 64% of IV FOS cases were responders compared to 22.2% of placebo cases (p=0.043). There was also a significant difference in the 2-hour responder rate,

with 95.4% responders in IV FOS group vs 55.5% in the placebo group (p=0.02). There were no significant differences in any adverse effects or in-hospital mortality rates between the two groups (p=0.63). IV FOS is safe and effective for the treatment of SE following IV DZP.

1.3.3 Translatability of Therapeutic Research between Human and Canine Status Epilepticus

Historically, animal models of epilepsy, typically induced chemically or by electrical stimulation, are used for the screening of potential antiseizure drugs. Although these have proven useful for the development of many first- and second-generation ASDs, they are not representative of human epilepsy or natural epileptogenesis. Rodent models of chronic epilepsy exhibit a low level of complexity regarding interindividual differences in cellular and molecular changes as well as genetic involvement (Potschka et al. 2013). Furthermore, these animal models are not able to predict for the approximate 30% of patients who develop pharmacoresistant epilepsy (Leppik 1992). Traditional animal models of epilepsy do not take into account the progression of epilepsy, or the development of drug resistance (Loscher et al. 1985). As a result, the current strategies for ASD development use models that are known to respond to presently marketed drugs, which may hamper the development of drugs with different mechanisms of actions that may be useful in treating pharmacoresistant seizures. There is a need for better animal models in the preclinical screening and assessment of ASD candidates (Potschka et al. 2013).

The ideal animal model of epilepsy has the following: development of spontaneously occurring recurrent seizures, a type of seizure similar in its clinical presentation to those occurring in human epilepsy, clinical seizures should be associated with epileptic-like activity in the electroencephalogram, pharmacokinetics of ASDs similar to those in people allowing for maintenance of effective drug concentrations, and effective plasma ASD concentrations similar to those reported for human epilepsy (Loscher 1984). As stated best by the British statistician George Box, “all models are wrong, some are useful.” It is likely that no model can meet all these criteria, but canine epilepsy may serve as a useful one for human epilepsy and has been compared to the human condition for decades (Barker 1973; Loscher et al. 1985; Patterson 2014a).

When evaluating animal models for their usefulness, one should consider whether the cause of disease is mirroring clinical etiology (i.e. etiological validity), whether their symptoms and pathology mimic those in people (i.e. face validity), and whether their response to therapy is similar to those in humans (i.e. predictive validity) (Potschka et al. 2013). As described, dogs have naturally-occurring epilepsy and SE with etiologies and pathology that closely mimic the human condition and are the only genetic animal model of epilepsy where interindividual variability exists that allows for the ability to select between animals that develop pharmacoresistant epilepsy and those that remain sensitive to currently marketed ASDs (Wolfgang Löscher 1997). In addition to breeds with suggested inherited idiopathic epilepsies, canine genetic studies have revealed genes found in canine progressive myoclonic epilepsies that are orthologous to

those causing human epilepsy syndromes (Ekenstedt, Patterson, and Mickelson 2012). For instance, the *LGI2* gene (an ortholog of the human epilepsy gene *LGI1*) in the Lagotto Romagnolo breed was found to cause recessive benign familial juvenile epilepsy (Jokinen et al. 2007; Seppälä et al. 2011) and the *EPM2* gene (an ortholog of human genes *EPM2A* or *EPM2B*) was discovered to cause Lafora disease in Miniature Wirehaired Dachshunds (Lohi et al. 2005). Similarly, causes for reactive seizures in dogs are comparable to those in humans, including but not limited to hypoglycemia, electrolyte disturbances, hyperthermia, and ethylene glycol poisoning (Brauer, Jambroszyk, and Tipold 2011; Levy 1994; Hutchinson et al. 2012; Bruchim et al. 2006; Keller and Goddard 2012). Finally, structural seizures in dogs, as they do in humans, occur following traumatic brain injury, inflammatory disease, neoplasia (Fredsø et al. 2017; Steinmetz, Tipold, and Löscher 2013).

The types of seizures and clinical presentation of seizures and SE in dogs are comparable to those observed in people, so much so that the terms used to describe canine seizures are adapted from human seizure terminology. As noted previously, seizures in dogs are categorized into three: focal, generalized and focal epileptic seizure evolving to become generalized. These are analogous to the focal, generalized, and focal to bilateral tonic-clonic seizures. Similarly, dogs present most often with generalized convulsive SE, but nonconvulsive SE has been observed when EEG is available. The largest difference in categorization is that awareness may be more difficult to assess in the canine patient. As a result, veterinary categorization of seizure types do not include impaired awareness

focal onset seizures, nor cognitive focal onset seizures (Berendt et al. 2015; Fisher et al. 2017). Furthermore, EEG presentation of seizures have been shown to be similar between dogs and people (Holliday, Cunningham, and Gutnick 1970; M. Berendt et al. 1999; Davis et al. 2011). In fact, seizure forecasting algorithms have been built and demonstrated 0.84 area under the classification curve using open access chronic ambulatory intracranial EEG from five dogs with naturally-occurring epilepsy and two humans undergoing prolonged intracranial EEG monitoring (Brinkmann et al. 2016). Finally, recent studies have reported blood-brain barrier (BBB) dysfunction in dogs with idiopathic epilepsy, along with evidence of altered neurogenesis, neuroinflammation, increased expression of P-glycoprotein, and hippocampal atrophy (Borschensky et al. 2012; Patterson 2013; Dirrig and Lamb 2016; Hanael et al. 2019; Pekcec et al. 2009; Kuwabara et al. 2010; Czerwik et al. 2018), pathology paralleling observations in epileptic foci of patients (Thom et al. 2005; Zhong, Ren, and Tang 2016; Marchi and Lerner-Natoli 2013; Feldmann et al. 2013; Blümcke et al. 2013).

Clinical studies conducted in canine SE have also shown to be useful in mirroring human responder rates. For the treatment of SE, studies comparing the effectiveness of IN MDZ and PR DZP have been conducted mostly in children (Bhattacharyya, Kalra, and Gulati 2006; Holsti et al. 2010, 2007; Fisgin et al. 2002; De Haan et al. 2010). In general, these studies considered a success as seizure termination within 10-15 minutes of drug administration and found that IN MDZ was at least as effective, if not superior, as PR DZP. In one pediatric study, effectiveness to stop prolonged seizure activity before emergency department

arrival without recurrence was compared between IN MDZ (using a Mucosal Atomization Device [MAD]) administered by paramedics and PR DZP historical controls (Holsti et al. 2007). Seizures recurred in the ED in 38% and 72% of patients in the IN MDZ and PR DZP groups, respectively. These results are similar to those seen in the IN MDZ (MAD) and PR DZP study in dogs in which the primary endpoint was seizure termination within 5 minutes without recurrence in 10 minutes in an emergency department setting (M. Charalambous et al. 2017). Seventy percent and 20% of cases had success, respectively, with a recurrence rate of 79% and 100%. For studies in established SE, IV FOS compared to placebo had a 64% 12-hr responder rate, and was similar to the 56%-69% success rate reported in the literature in people (Patterson et al. 2015; Treiman et al. 1998; Gujjar et al. 2017; Chakravarthi et al. 2015; Mundlamuri et al. 2015). Furthermore, Patterson et al demonstrated that this response rate was achieved at the same unbound plasma FOS concentration range that had been demonstrated to be therapeutic in humans. These results further supported the observation that dogs have similar responses to ASDs as humans, and the translation of therapeutic and mechanistic research between canine and human epilepsy.

1.3.3.1 Advantages of Using Canine SE as a model of Human SE

There are several advantages to using canine SE as a translational platform for therapeutic and mechanistic research between preclinical rodent models and human SE. First, they are large enough to evaluate behavioral responses and to

accommodate human devices (Potschka et al. 2013). Second, as noted in the previous sections, there are many similarities in clinical and neurophysiological presentation of SE in dogs and people (Patterson 2014a). The presence of nonconvulsive and convulsive SE, and the subdivision of nonmotor and motor focal seizures is astonishing, as are the similarities in epileptiform and epileptic activity in EEG (Davis et al. 2011). Next, their response rate to therapies approved to treat human SE syndromes are comparable, and as a result, many of the drugs used to treat canine SE are also used to treat human SE, including using BZDs as a first-line agent in SE treatment guidelines (Glauser et al. 2016; Blades Golubovic and Rossmeisl 2017b).

1.3.3.2 Limitations of Using Canine SE as a model of Human SE

One important limitation of using canine SE as a model for human SE is we cannot interview canine patients and are restricted to pet owner recounts of description and frequency SE episodes (Mette Berendt et al. 2015). Second, although NCSE is often diagnosed using EEG in people, scalp EEG is not routine nor practical in canine patients, because animals are often moving excessively, and only way to get a good reading is to sedate them which leaves an EEG artifact on its own. Furthermore, canine skulls are surrounded by a layer of muscle, which could also leave electrical artifacts (Potschka et al. 2013). Another key limitation is that some owners decline diagnostic investigation of SE due to financial concerns (Mette Berendt et al. 2015), which may contribute to inaccurate diagnosis and epidemiological findings of canine SE. A few

consequences of the natural occurrence of seizures and SE in canines is that the investigators are unable to elicit seizures and SE to screen potential therapeutic agents, it is much more difficult to recruit for canine clinical trials than it would be to screen ASDs in rodent models. In addition, it is much more costly in time and money and not pragmatic to select for and breed epileptic sublines of dogs to have enough subjects for experimental study (Loscher and Meldrum 1984). Finally, dogs metabolize drugs differently than people do. In general, drugs are metabolized at a faster rate in dogs compared to humans, and different pharmacokinetics would have to be considered for potential therapies (Frey and Löscher 1985). However, this can be overcome by targeting therapeutic drug concentrations rather than doses. Dogs may also have different metabolite profiles, which may prohibit them from using specific drugs and limit translation to humans (Dalgaard 2015; Martignoni, Groothuis, and de Kanter 2006; Yoshida et al. 2018).

CHAPTER 2

DEVELOPMENT OF ALLOPREGNANOLONE FOR THE EARLY TREATMENT OF STATUS EPILEPTICUS

2.1 Introduction

In this chapter, I will discuss the development of intravenous and intramuscular allopregnanolone for early treatment of convulsive SE. The central hypothesis is that ALLO would be beneficial in the early treatment of SE based on its novel mechanisms of action and ability to rapidly diffuse into the brain. The specific aim of my study was to characterize the pharmacokinetics (PK), pharmacodynamics (PD) and safety/tolerability following IV and IM ALLO in dogs. The objectives of these studies were to 1) develop PK models that describe concentration-time profiles following single ascending doses of IV and IM ALLO, 2) sequentially build a PK-PD model to describe the plasma concentration-intracranial EEG data, and 3) assess the safety and tolerability of IV and IM ALLO. First, I will provide a brief review of ALLO and a rationale for the development ALLO for the treatment of SE, followed by a summary of the studies conducted in the following order: IV ALLO PK and safety, IM ALLO PK and safety, and PK-PD modeling. The last section describes the design of a clinical trial of IV ALLO for the treatment of CSE.

2.2 Review of Allopregnanolone

2.2.1 Endogenous Allopregnanolone

Allopregnanolone is a progesterone derivative that is produced in the brain, adrenals, and gonads (Reddy and Rogawski 2012; Corpéchot et al. 1981). In men and women, the steady-state plasma ALLO concentration is less than 2 ng/mL (Pierucci-Lagha et al. 2006; Girdler et al. 2001; Ottander et al. 2005;

Droogleever Fortuyn et al. 2004). However, plasma ALLO concentrations have been shown to increase in response to stress, menstruation, and throughout each trimester of pregnancy (Purdy et al. 1991; Droogleever Fortuyn et al. 2004; Reddy 2009; Herzog 2015; Luisi et al. 2000). It functions as an endogenous anxiolytic and uterine myorelaxant via $GABA_A$ modulation (Bali and Jaggi 2014; Putnam et al. 1991). In healthy, pregnant women, ALLO levels have been reported to reach as high as 150 nM in the third trimester (Luisi et al. 2000).

2.2.2 Physicochemical Properties

Allopregnanolone (molecular formula $C_{21}H_{34}O_2$) has a molecular weight of 318.5 g/mol and a topological polar surface area of 37.3 A^2 (National Center for Biotechnology Information 2019a). With a logP of 4.9, it is insoluble in water, slightly soluble in methanol, soluble in 2-methyl-tetrahydrofuran, and freely soluble in tetrahydrofuran (National Center for Biotechnology Information 2019a; FDA CDER Other Review(s) 2019). The low molecular weight, small polar surface area and lipophilicity suggests it would easily cross biological membranes, namely the blood-brain barrier. These are ideal characteristics for a drug intending to treat seizure emergencies. Its generic name, provided by the United States Adopted Names Council, is brexanolone and will be used interchangeably with allopregnanolone throughout this chapter.

2.2.3 Known Mechanisms of Action

Allopregnanolone is a positive allosteric modulator of $GABA_A$ receptors, and binds on the α-subunit and in the interface between α- and β-subunits, enabling potency at synaptic and extrasynaptic $GABA_A$ receptors (Hosie et al. 2006). At nanomolar concentrations (300 nM), ALLO potentiates GABA currents and requires GABA for chloride channel activation, while at micromolar (1 μM) concentrations, it functions as a direct agonist of the channel (Majewska et al. 1986). ALLO has also been shown to have neuroprotective effects in traumatic brain injury, ischemia, and neurodegenerative diseases models (He et al. 2004; He, Hoffman, and Stein 2004; Irwin, Solinsky, and Brinton 2014; Irwin et al. 2015; Djebaili, Hoffman, and Stein 2004; Moralí et al. 2011; Napoli et al. 2019; Mellon, Gong, and Schonemann 2008). In addition, the neurosteroid has been shown to increase superoxide dismutase 2 enzyme (decreases neuronal death as a result of reactive oxygen species production) following pilocarpine-induced SE in mice and increase phosphorylation and membrane insertion of extrasynaptic $GABA_A$ receptors via protein kinase C (Cho et al. 2018; Lejri et al. 2017; Abramian et al. 2014; Modgil et al. 2017).

2.2.4 Clinical Pharmacokinetics

Brexanolone has low oral bioavailability of <5% (FDA CDER 2019). Its volume of distribution is approximately 3 L/kg and is highly plasma protein bound (>99%) (ZULRESSO™ [package insert] 2019). With an estimated total clearance of 1 L/h/kg, its terminal half-life is approximately 9 hours(ZULRESSO™ [package

insert] 2019). Brexanolone is extensively metabolized by non-cytochrome P540 (CYP) pathways, including aldo-keto-reduction, glucuronidation, and sulfation (FDA CDER 2019). The three major metabolites in humans are sulfated or glucuronidated conjugates of the C20-reduced form of brexanolone, and are not pharmacologically active (FDA CDER 2019). Less than 1% of brexanolone is excreted via urine or feces unchanged (FDA CDER 2019). Due to its extensive metabolism via multiple pathways, the sponsor claims that brexanolone is less susceptible to metabolic drug-drug interactions (FDA CDER 2019). *In vitro* studies suggested that brexanolone showed potential to inhibit CYP2C9. However, co-administration with phenytoin, a CYP2C9 substrate, did not result in clinically significant changes in phenytoin pharmacokinetics (ZULRESSO™ [package insert] 2019).

In dogs, brexanolone is rapidly and extensively metabolized to produce 48 quantifiable compounds (FDA CDER 2019). Oxidation was the exclusive biotransformation mechanism identified in dogs (FDA CDER 2019). Only a minimal amount of the parent compound was excreted in the bile, urine, and feces (FDA CDER 2019).

2.2.5 FDA-Approved Indications and Marketed Formulations

Brexanolone is indicated for the treatment of moderate-to-severe post-partum depression (ZULRESSO™ [package insert] 2019). It is marketed as a clear, colorless, and preservative-free injectable solution. One milliliter of ZULRESSO™

contains 5 mg brexanolone, 250 mg of betadex sulfobutyl ether sodium, 0.265 citric acid monohydrate, 2.57 mg sodium citrate dihydrate, and water.

2.2.6 Present State of Knowledge of Allopregnanolone in Status Epilepticus

Allopregnanolone has been shown to be effective in terminating seizures in several SE rodent models, including pilocarpine, kainate, perforant path stimulation, and tetramethylenedisulfotetramine (Rogawski et al. 2013; Kokate et al. 1996; C A Frye 1995; Cheryl A Frye and Scalise 2000; Zolkowska, Wu, and Rogawski 2018). Clinically, ALLO has only been studied in super-refractory SE (Vaitkevicius et al. 2017; Broomall et al. 2014; Rosenthal et al. 2017; Sage Therapeutics 2015). An open-label Phase I/II study in adult and pediatric (≥2 years) patients with SRSE was conducted using a brexanolone loading dose (286.6 μg/kg for 1 hour), followed by a four-day maintenance infusion (standard: 86 μg/kg/hr or high-dose: 156 μg/kg/hr) and one-day taper period (Rosenthal et al. 2017). The inclusion criteria included patients who failed to respond to at least one first-line agent, one second-line agent, and who were unable to be weaned off of third-line agents (TLAs) after ≥24 hours or had breakthrough seizures after ≥6 hours of initiation of burst-suppression from third-line therapies. The standard maintenance infusion rate was chosen to attain a steady-state concentration of 150 nM (approximately 50 ng/mL), which is the physiological maximum plasma ALLO concentration observed in healthy pregnant women (Luisi et al. 2000; Broomall et al. 2014; Vaitkevicius et al. 2017). Responders were defined as patients who were successfully weaned from TLAs without reinitiation of other

TLAs during administration of brexanolone. Brexanolone administration was well-tolerated, with response rate of 77% (n=17/22, 13 standard dose, 4 high-dose) and improved overall functional status measured at follow-up day 29.

This success was followed by a failed Phase III placebo-controlled trial of brexanolone for SRSE in 132 adult and pediatric (≥2 years) patients (Sage Therapeutics 2015). Similar to the Phase I/II trial, brexanolone was administered as a 300 μg/kg loading dose in the first hour, followed by a constant rate infusion at 90 μg/kg/hr, followed by a slow taper off brexanolone in the last 24 hours. In contrast to the Phase I/II study, the brexanolone infusion was designed to last one additional day, the inclusion criteria required patients to fail a qualifying wean (even if they had failed at least one wean prior to screening), there was an open-label high-dose arm (150 μg/kg/hr for 119 hours) if the patient failed the primary endpoint, and the response was defined with more strict criteria (i.e. no reinitiation of TLAs within 24 hours following study drug infusion). Brexanolone was no different than placebo in meeting the primary endpoint (43.9% vs 42.4%, respectively). The investigators found that during the screening of the Phase III study, 52% (144/276) of patients were able to successfully wean off of their TLAs. It is possible given the differences in study design and inclusion criteria that the patients in the Phase III study were harder to treat than those included in the Phase I/II study. Further, the lack of control arm made it difficult to estimate the placebo response. In addition, nearly 40% of the study population (27 in placebo arm, 24 in brexanolone arm) opted into the open-label high-dose arm. It is possible that clinicians may have been tempted to declare an early failure in

order for their patient to receive “active” drug. Finally, the targeted steady-state concentration was based off of the highest observed concentration healthy, pregnant women, which may be safe but not sufficient for treating SE. Nonetheless, the results of this Phase III trial provides a number of considerations and lessons for future development, including whether the SRSE population is where brexanolone use could be most beneficial.

One reason why brexanolone would not be an ideal choice to treat SRSE is that its main mechanism of action is equivalent to those used by TLAs to induce burst/seizure suppressions, i.e. via $GABA_A$ receptor potentiation. In the Phase III study, SRSE was defined when patient experienced breakthrough seizures during the induction of burst/seizure suppression or when a patient had breakthrough seizures while attempting a wean from TLAs. Since an anesthetic agent will inevitably induce seizure suppression if the dose is high enough (Shorvon and Ferlisi 2012), the more common diagnosis of SRSE was likely due to the latter reasoning (per personal communication with Dr. M. A. Rogawski, August 2019). Physical dependence is a physiological phenomenon that occurs with CNS-acting drugs, including those that act via $GABA_A$ receptors (e.g. ethanol, benzodiazepines, barbiturates) (Boisse et al. 1990; Okamoto, Hinman, and Aaronson 1981; Rosenberg and Chiu 1985; Miranda and Pinardi 1998; Cicero et al. 1971). Following chronic administration and subsequent abrupt withdrawal of $GABA_A$-potentiating drugs, rebound conditions of the symptoms treated originally with the drug (such as seizures or anxiety) occur. Therefore, one could hypothesize that following chronic seizure suppression with a TLA

following by the administration of brexanolone, patients would have a high risk of rebound seizures. A monotherapy or combination of therapies with at least one different mechanism of action (i.e. not via $GABA_A$ receptor potentiation) may have a higher likelihood of success for the treatment of SRSE.

2.2.7 Rationale for Developing Allopregnanolone for Early Treatment of SE

In addition to having activity at synaptic $GABA_A$ receptors, like the current first-line therapies, ALLO has exhibited additional mechanisms of action that would be beneficial in terminating SE, including activity at extrasynaptic $GABA_A$ receptors which mediate tonic inhibition, and insertion of additional extrasynaptic $GABA_A$ receptors (Hosie et al. 2006; Abramian et al. 2014; Modgil et al. 2017). Similar to benzodiazepines, ALLO has ideal physicochemical properties (low molecular weight and high logP) that enable its rapid distribution into the brain (Irwin et al. 2015). The rapid brain penetration also manifests as a fast onset of action of sedative (within 5 minutes following IM injection and 30 seconds following IV injection) and antiseizure effects (within 1 minute of intraperitoneal injection) in rodent models (Irwin et al. 2015; Rogawski et al. 2013). ALLO, at 2- and 3-mg/kg given intraperitoneal and intramuscularly, respectively, has shown preclinical efficacy in seizure termination following drug-induced SE in early and benzodiazepine-refractory stages (Rogawski et al. 2013; Zolkowska, Wu, and Rogawski 2018).

The development of ALLO for treatment of SE is currently limited by its failure in SRSE. Evaluating the safety and effectiveness of drugs in the SRSE

patient population is particularly difficult and ethically complex given that these patients 1) are the smallest subset of patients experiencing SE, 2) are critically-ill and have the highest morbidity and mortality risk (and consequently, a higher futility risk), and 3) there are no widely-accepted standards of practice to compare to active drug arm or even standard practices between clinical sites. Therefore, developing ALLO for early termination of SE would have the advantage of inclusion of active, standardized comparators, larger patient population, and a lower risk of confounding medical complications. Moreover, due to the desire to quickly attain peak brain concentrations and/or the ability to treat SE in the pre-hospital setting with relative ease, pursuing the IV and IM routes of administration would be ideal.

2.3 Pharmacokinetics and Safety of Intravenous Allopregnanolone for Early Treatment of Status Epilepticus in Dogs

2.3.1 Introduction

The central hypothesis is that ALLO possesses the requisite pharmacologic, physicochemical, and pharmacokinetic (PK) properties to serve as an early treatment for SE, either in combination with BZDs or as a replacement. One approach to testing this hypothesis is the use of dogs with naturally-occurring epilepsy, which is similar to human disorder in its electroencephalographic presentation and response to therapy (Chapter 1.3). Although IV ALLO pharmacokinetics has been characterized in rodents and humans (Irwin et al. 2015; Luisi et al. 2000), there is a lack of PK data in dogs.

My working hypotheses are 1) ALLO exhibits linear pharmacokinetics with respect to dose, and 2) ALLO is safe to administer IV. The specific aim of this study is to characterize IV ALLO PK and safety following single ascending doses. The primary objectives were to develop a PK model to describe the concentration-time data, evaluate its safety and tolerability, and simulate dosing regimens that can be used to inform dosing recommendations for a clinical study of CSE.

2.3.2 Methods

2.3.2.1 Study Animals and Safety Monitoring

Five dogs with (n=2) and without (n=3) a history of seizures were used. One of the dogs with a history of seizures had recurrent seizures despite being on phenobarbital (PB) maintenance regimen, while the other dog had not had seizures in the last 7 years and was not on an ASD. Approval to conduct the study was obtained through the Institutional Animal Care and Use Committee of the University of Minnesota. The dogs were housed at the University of Minnesota's College of Veterinary Medicine. The dog on PB was previously implanted with a device which wirelessly transmits continuous iEEG recordings (Kremen et al. 2018). On study days, each dog participating in the study was removed from their kennel to have a central-line catheter inserted an hour prior to study start. On days where IV ALLO was administered, a peripheral-line catheter was also inserted. Drug administration took place in a procedure room away from the dog's kennel.

2.3.2.2 Study Drug

ALLO concentrate was provided by the Rogawski laboratory at the University of California, Davis. The concentrate consists of 6% allopregnanolone in 24% sulfobutyl ether β-cyclodextrin (Dexolve™) in a 0.9% sodium chloride solution (normal saline). The concentrate was prepared using chemically pure, laboratory grade ALLO using Good Manufacturing Practices and shipped frozen to the University of Minnesota. Frozen ALLO was thawed and diluted with normal saline (1 part ALLO**:** 1-3 parts normal saline, as directed by the manufacturing laboratory) prior to IV administration for a final ALLO concentration of 1.5-3 mg/mL.

Dexolve™ (CycloLab) is a solubilizing agent comprised of a ring of a hydrophilic exterior and lipophilic interior environment and is considered a "generic" of the cyclodextrin Captisol® (Ligand). Both are cyclic oligosaccharrides with a sodium sulfonate salt separated from the ring structure by a butyl ether group (Captisol®). The cyclodextrin is able to dissolve the poorly water-soluble compounds via noncovalent interactios within the lipophilic cavity (Loftsson and Brewster 1996; Varan et al. 2017). Captisol® is exclusively renally eliminated and has been shown to be safe in humans. The technology has received FDA approval for its use in parenteral formulations of carbamazepine, amiodarone, and voriconazole.

2.3.2.3 Starting Dose Rationale

Basal levels of ALLO in healthy, pregnant women have been reported to reach as high as 50 ng/mL (Luisi et al. 2000). Using allometric scaling of preclinical and clinical data reported in the literature (Tables 2.3.2-1 and 2.3.2-2), PK

Table 2.3.2-2 PK parameter values reported in the literature

Species	Wt (kg)	Dose (mg/kg)	Cmax (ng/mL)	AUC_{inf} (hr*ng/mL)	k_{el} (hr^{-1})	$t_{1/2}$ (hr)	Vd (L/kg)	Cl (mL/hr/kg)
Mouse[1]	*0.03*	1.5	214.6	155.6	0.174	4	55.4	9640.1
Rabbit[1]	3.6-4.5	3	1176	625.1	0.209	3.3	23	4799.2
Human (female)[2]	*70*	Cumulative 0.09 mg/kg		47.15	0.156	4.35	12.5	1956

[1]Irwin et al 2015, [2]Timby et al 2006

Table 2.3.2-1 Estimated and Calculated Three-compartment Model PK Parameter Values.

	Volume of distribution (mL/kg)			Clearance (mL/(kg*min)		
Species	V	V2	V3	CL	CL2	CL3
Rat	0.15	1527	1944	101.2	3275	72.94
Dog	0.06	598.3	761.8	38.09	1233	27.47

Rat data provided by the Rogawski Lab. Dog PK parameters calculated by allometric scaling using preclinical and clinical data in Table 2.3.2-1.

parameters for dogs were determined. These parameter estimates were used to simulate concentration-time profiles and determine doses and dose regimens predicted to attain plasma concentrations in the 50 ng/mL range for approximately an hour following infusion (Figure 2.3.2-1). A 1 mg/kg dose infused over 5 minutes was chosen based on my simulations.

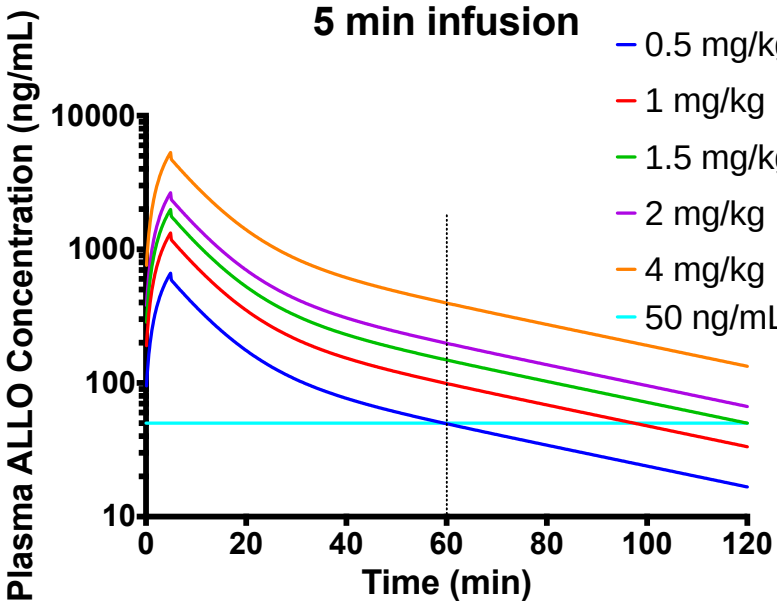

Figure 2.3.2-1 Dosing Simulations for Initial IV ALLO Dose in Dogs

2.3.2.4 Study Design

Three healthy dogs and one dog with a history of seizures were given single IV doses of ALLO ranging from 1-4 mg/kg as a 5-minute infusion via a catheterized peripheral vein (Table 2.3.2-3). One to two dogs were studied at each dose. Whole

Table 2.3.2-3 Number of Animals per IV Study Dose

Route	IV			
Dose (mg/kg)	1	2	3	4
Number of Dogs (total)	4	4	2	2
Number of Dogs on PB	2	2	0	0
Number of Dogs with EEG	1	1	0	0

blood samples (~2 to 5 mL) were collected via a catheterized central vein at pre-dose and at approximately 3, 5, 15, 30, and 45 minutes, 1, 2, 4, and 6 hours post-infusion. There was a washout period of at least 1 week.

After completion of the IV dose escalation study, two healthy dogs were given a standard PB dose (2 mg/kg) until steady state was reached (at least 15 days). Once at steady state, the ALLO PK study was repeated in these dogs and

one dog with a history of seizures on chronic PB (1-2 mg/kg). PB was discontinued in both healthy dogs immediately after IV study completion.

2.3.2.5 Allopregnanolone Assay

Whole blood was collected in EDTA-containing purple-top vacutainer tubes, and centrifuged for plasma separation. The red blood cells and plasma were immediately frozen (−80°C) until analysis. An ultra high-performance liquid chromatography-mass spectrometry (UPLC-MS/MS) method developed and validated at the UC Davis laboratory was used to measure total plasma ALLO concentrations (Zolkowska, Wu, and Rogawski 2018).

2.3.2.6 Pharmacokinetic Analysis

2.3.2.6.1 Non-compartmental Analysis

ALLO concentration–time data were analyzed using non-compartmental analysis (Phoenix 64, Build 8.0.0.3176, Certara L.P., Princeton, NJ, USA). Data was uniformly weighted. Pharmacokinetic parameters determined included first observed concentration (C_1), terminal rate constant (k), and terminal half-life ($t_{1/2}$). k was calculated using WinNonlin default setting, including estimating k using a regression with the largest adjusted R^2 value with the largest number of concentration-time points used. Each individual concentration-time profile was visually checked to make sure the concentration-time data points were adequate and sufficient concentration-time points to characterize k. If not, user-defined concentration-time points were used. $t_{1/2}$ was calculated as 0.693/k. The area

under the time–concentration curve from time 0 to infinity (AUC_∞) was calculated using the linear up log down method and equation *(1),* where Cp is the plasma ALLO concentration, t_{last} is the time at which the last plasma sample was measured, Cp_{last} is the last measured plasma ALLO concentration, and k_{el} is the terminal rate constant. Clearance (CL) and volume of distribution (Vd) were calculated using equations *(2)* and *(3)*, respectively, where the bioavailability (*F*) is assumed to be 100% for an IV dose. Dose proportionality was determined by comparing log-normalized AUC_∞ across all doses using a one-way ANOVA test. Concentration–time profiles were created using the GraphPad Prism 7 (Version 7.0a, GraphPad Software, Inc., La Jolla, CA, USA).

Equations:

$$AUC_\infty = AUC_{0-t_{last}} + AUC_{t_{last}-\infty} = \int_0^{t_{last}} C_p dt + \frac{C_{p_{last}}}{k_{el}} \quad (1)$$

$$CL = \frac{Dose * F}{AUC_\infty} \quad (2)$$

$$CL = k_{el} * V_d \quad (3)$$

2.3.2.6.2 Compartmental Analysis

PK parameters estimates were also determined using compartmental analysis (Phoenix Non-Linear Mixed Effects 8.0). PK parameter values were estimated from individual concentration-time profiles using a first-order conditional estimation extended least squares method. Additive, multiplicative, and combined error models for residual unexplained variability were evaluated. The best fit model was determined using visual inspection, goodness of fit plots (e.g.

conditional weighted residual plots and individual observed versus predicted), and precision of model parameters. Arithmetic and geometric means and standard deviation of parameter values were calculated using Phoenix Descriptive Statistics function.

A population approach was used to determine PK parameter "typical values." An exponential error model for between-subject variability was used. Additive, multiplicative, and combined error models for residual unexplained variability were evaluated. The best fit model was determined as stated above, with the addition of evaluating objective function value (OFV, as determined by the log-likelihood function) and Akaike's Information Criterion.

Normalized weight with fixed allometric exponents of 0.75 and 1 was added as a covariate on clearance and volume, respectively. Weight was normalized to median weight. The relationship of the covariate and PK parameter was modeled by the equation $PK = (\frac{WT}{19.2})^{0.75\ or\ 1} * tvPK * e^{\eta PK}$, where PK is the PK parameter, tvPK is the typical value of that parameter from the population, dPK is the estimated value of the covariate effect, and ηPK is the between-subject variability of that parameter.

Pharmacokinetic parameter estimates from the population model were used to simulate concentration-time profiles from a "typical dog" with a weight of 19.2 kg (median weight of dogs in my study) receiving a single 5-minute infusion of 1-4 mg/kg ALLO.

2.3.2.7 Safety and Behavioral Response Evaluations

A modified Glasgow coma scale (mGCS) was used to quantitate degree of sedation pre-dose and at scheduled blood sampling times (Table 2.3.2-4). Cardiorespiratory activity (blood pressure, heart rate, and respiratory rate) was assessed at blood sampling times up to 30 minutes. In addition to sedation and vitals, the dogs were monitored for vomiting, diarrhea, and lethargy prior to and for 60 minutes after drug administration, and at each blood sampling time. Behavioral and iEEG activity were monitored by veterinary staff for seizure activity. As doses were escalated, the maximal tolerated toxicity, as determined by the supervising veterinarian, was 20 minutes of sedation with stable cardiorespiratory activity (respiratory rate greater than 6 or less than 60 bpm, systolic blood pressure greater than 60 mmHg, heart rate greater than 50 but less than 160 bpm).

Table 2.3.2-4 Modified Glasgow Coma Scale. Reproduced with permission from Platt et al 2001.

	Score
Motor activity	
Normal gait, normal spinal reflexes	**6**
Hemiparesis, tetraparesis, or decerebrate activity	**5**
Recumbent, intermittent extensor rigidity	**4**
Recumbent, constant extensor rigidity	**3**
Recumbent, constant extensor rigidity with opisthotonus	**2**
Recumbent, hypotonia of muscles, depressed or absent spinal reflexes	**1**
Brain stem reflexes	
Normal pupillary light reflexes and oculocephalic reflexes	**6**
Slow pupillary light reflexes and normal to reduced oculocephalic reflexes	**5**
Bilateral unresponsive miosis with normal to reduced oculocephalic reflexes	**4**
Pinpoint pupils with reduced to absent oculocephalic reflexes	**3**
Unilateral, unresponsive mydriasis with reduced to absent oculocephalic reflexes	**2**
Bilateral, unresponsive mydriasis with reduced to absent oculocephalic reflexes	**1**
Level of consciousness	
Occasional periods of alertness and responsive to environment	**6**
Depression or delirium, capable of responding but response may be inappropriate	**5**
Semicomatose, responsive to visual stimuli	**4**
Semicomatose, responsive to auditory stimuli	**3**
Semicomatose, responsive only to repeated noxious stimuli	**2**
Comatose, unresponsive to repeated noxious stimuli	**1**

2.3.3 Results

The demographics of the dogs are listed in Table 2.3.3-1.

Table 2.3.3-1 Animal Demographics

ID	*Age (years)*	*Gender*	*Weight (kg)*	*Breed*	*Seizure Type*	*Seizure Frequency*	*Co-medications*
3	9	Male, neutered	16	Beagle	History of one witnessed seizure	None	None
6	13	Male, neutered	16	Keeshound Mix	Focal, with generalized seizures	Focal cluster seizures every 14-60 days. With secondarily generalized seizures every 1-2 months.	Phenobarbital (PB)
7	1	Female, intact	17	Coonhound Mix	(healthy)	None	None
8	1	Female, intact	22	Coonhound Mix	(healthy)	None	None
9	1	Male, intact	22	Coonhound Mix	(healthy)	None	None

2.3.3.1 Non-compartmental PK Analysis

The concentration-time profiles of IV are shown in Figures 2.3.3-1.

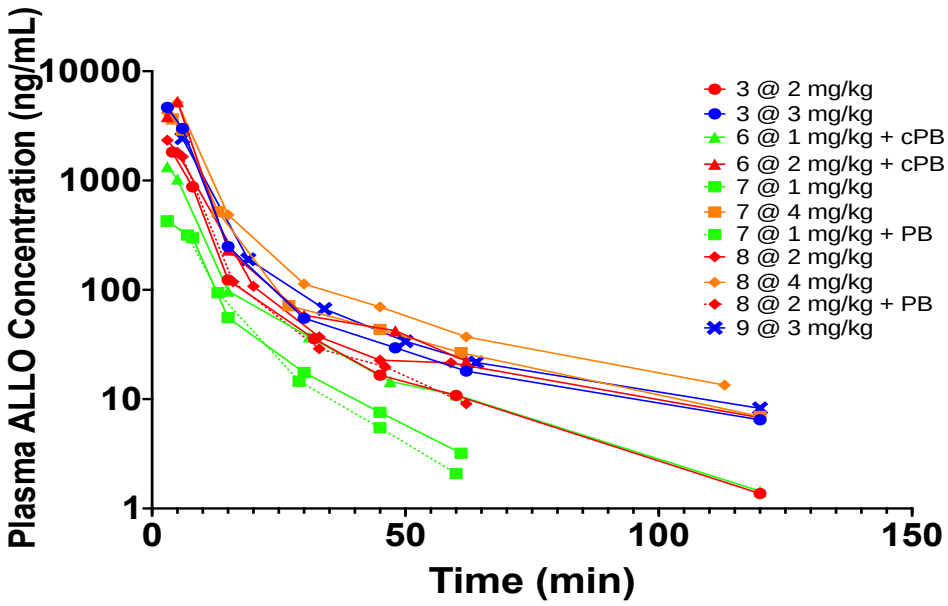

Figure 2.3.3-1 Plasma ALLO Concentration-Time Profile Following IV Administration on a log-scale. cPB: Chronic PB

Pharmacokinetic parameter estimates using noncompartmental analysis are summarized in Table 2.3.3-2. Following IV dosing, clearance ranged from 3.4-20.1 L/hr/kg, while volume of distribution ranged from 1.9-6.0 L/kg. The terminal half-life ranged between 7-33 minutes.

Table 2.3.3-2 Non-compartmental PK Parameter Estimates

Dose (mg/kg)	***n***	***λ_z (hr^{-1})***	***$t_{1/2}$ (hr)***	***C_{max} (ng/mL)***	***CL (L/hr/kg)***	***V (L/kg)***	***AUC_∞/Dose (ng/mL*hr/(ng/kg)) x 10^{-5}***
1	2 (1)	7.2-8.1 (3.6)	0.09-0.10 (0.2)	425-1340	12.1-14.2 (5.6)	1.5-2.0 (1.7)	7.0-8.2 (16.9)
2	3 (1)	1.2-4.0 (1.8)	0.17-0.56 (0.4)	1654-5287	6.2-9.5 (3.2)	2.0-5.0 (1.8)	10.4-12.0 (31.1)
3	2	2.1-2.3	0.31-0.33	2440-4661	6.2-7.9	2.9-3.4	12.6-16.1
4	2	1.4-1.7	0.40-0.50	3660-5172	5.9-8.2	4.0-4.6	12.2-17.9

Estimates are presented as a range. Values in parentheses are from one dog on chronic PB. λ_z: terminal phase slope; $t_{1/2}$: terminal half-life; C_{max}: observed peak plasma concentration; CL: clearance; V: volume of distribution; AUC_∞/Dose: dose-normalized area under the concentration-time curve from time 0 extrapolated to infinity

As shown in Figures 2.3.3-2 and 2.3.3-3, dose-normalized $AUC_{0\text{-}INF}$ and observed C_{max} across doses were similar.

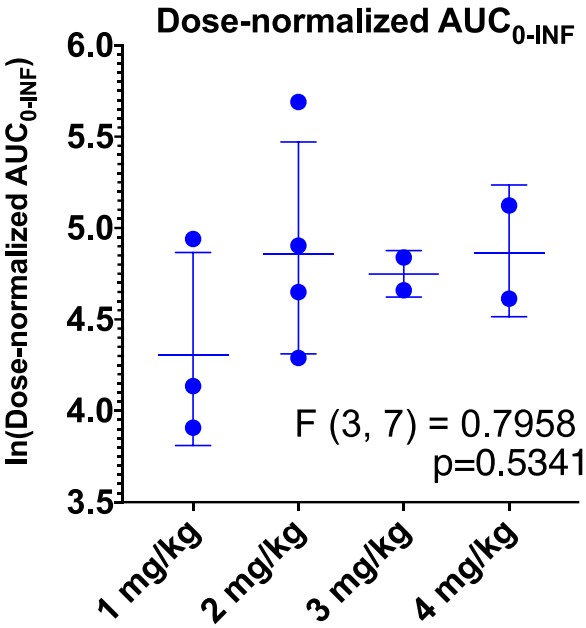

Figure 2.3.3-2 Dose-proportionality: Dose-normalized $AUC_{0\text{-}INF}$

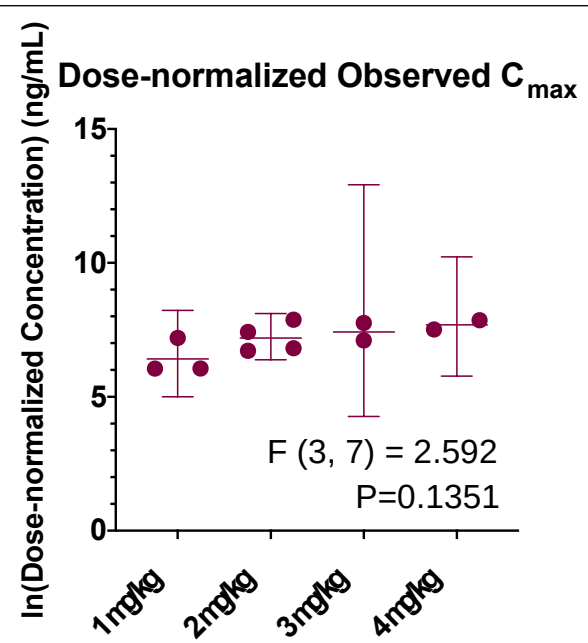

Figure 2.3.3-3 Dose-proportionality: Dose-normalized observed C_{max}

2.3.3.2 Individual Compartmental PK Analysis Following IV Administration

A two-compartment model with first-order elimination and a combined error model best fit all ALLO concentration data following IV administration (goodness of fit plots included in Figure 2.3.3-4). The model was unable to estimate PK

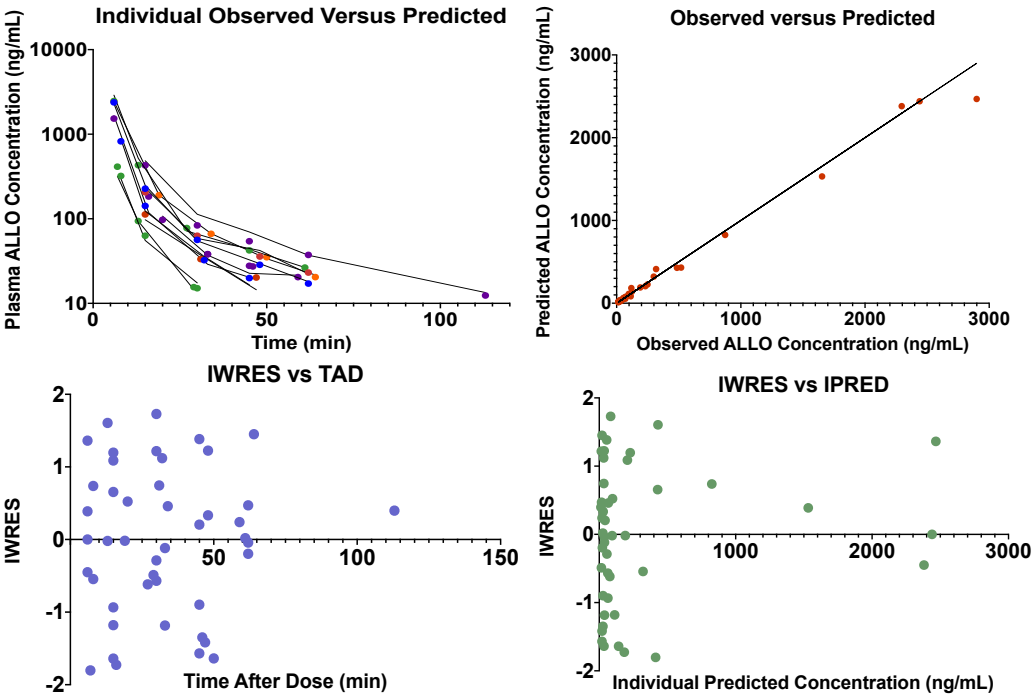

Figure 2.3.3-4 Goodness of fit plots for a two-compartment model. IWRES: individual weighted residual; TAD: time after dose; IPRED: individual predicted concentration

parameter values with precision for one animal, 6J. This may be due to the small number of concentration-time data above the lower limit of quantitation available for that animal. However, goodness of fit plots do not show gross signs of model misspecification. Individual and averaged parameter estimates are summarized in Table 2.3.3-3.

Table 2.3.3-3 Individual Compartmental PK Parameter Estimates

ID	*CL (L/hr)*	*Q (L/hr)*	*V (L)*	*V2 (L)*	*Proportional Error (%)*	*Additive Error (ng/mL)*
3G	122.3 (5.5%)	19.3 (11.6%)	7.7 (10.3%)	7.5 (14.1%)	8.0 (31.8%)	1.4 (100%)
6J	6.3 (294%)	0.1 (586%)	0.2 (348%)	0.1 (582%)	11.1 (45%)	3.4 (69.5%)
7D	180.8 (6.7%)	33.8 (14.1%)	12.9 (13.3%)	15.9 (21.8%)	12.8 (21.7%)	0.002 (---)
8N	155.2 (13.9%)	29.8 (28.6%)	13.0 (24.9%)	18.9 (29.5%)	20.5 (20.4%)	0.002 (---)
9B	171.7 (0.2%)	32.6 (1.8%)	13.5 (0.8%)	11.5 (2.5%)	---	0.8 (31.6%)
Mean (SD)	127.3 (71.2)	23.1 (14.1)	9.4 (5.7)	10.7 (7.4)	10.5 (7.5)	1.1 (1.4)
Geometric Mean (SD)	81.9 (0.3)	9.1 (0.8)	4.9 (0.01)	4.2 (0.01)	---	---

Values are shown with coefficient of variation (%). CL: clearance from central compartment; Q: intercompartment clearance; V: volume of distribution from central compartment; V2: volume of distribution from peripheral compartment.

2.3.3.3 Population Compartmental PK Analysis Following IV Administration

A population approach was also used to fit the IV concentration-time data. A two-compartment model with first-order elimination with proportional error model best

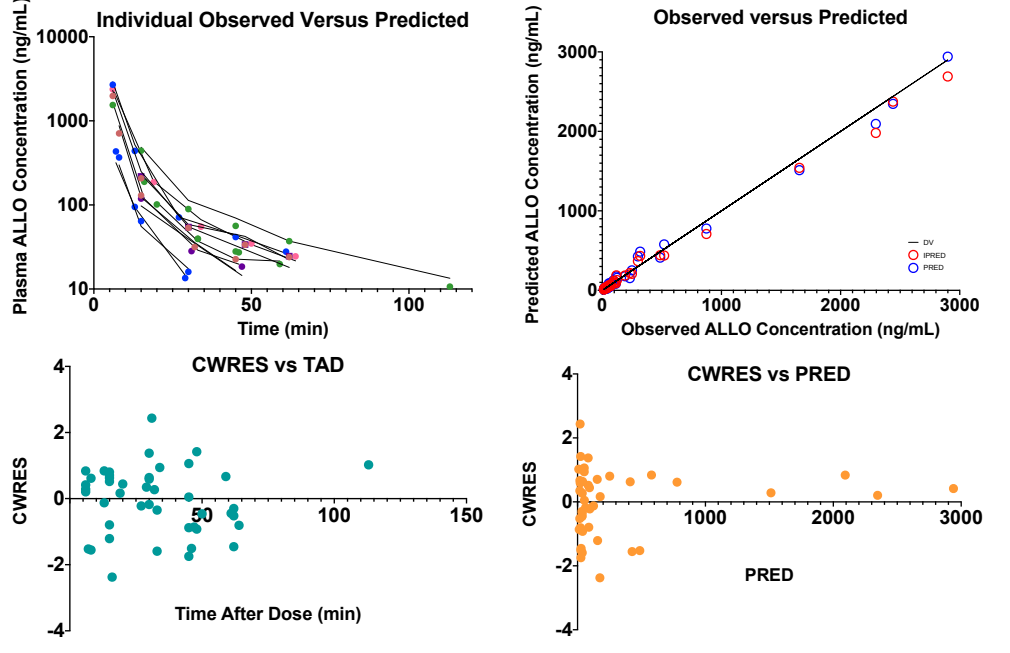

Figure 2.3.3-5 Goodness of fit plots for a population two-compartment Model. IPRED (red open circle): individual predicted concentration; PRED (blue open circle): population predicted concentration; CWRES: conditional weighted residual; TAD: time after dose

described all of the IV data (Figure 2.3.3-5). Goodness of fit plots do not show signs of model misspecification. PK parameter estimates and individualized PK parameter estimates (post-hoc) are summarized in Tables 2.3.3-4 and 2.3.3-5. Between-subject variability on CL was estimated to have a variance of 0.011. I was unable to estimate between-subject variability of volume without high eta shrinkage. For that reason, it was excluded from the final model. Allometric scaling on CL and V centered on the median weight improved the fit of the model (decrease in OFV of 8.0597) and were included in the final model. A proportional error model was used to describe the residual unexplained variability.

Table 2.3.3-4 Population Compartmental PK Parameter Estimates

MODEL PARAMETER		***ESTIMATE***	***STD. ERROR***	***CV%***	
FIXED EFFECT	V (L)	11.40	0.22	1.94	
	V2 (L)	16.15	2.17	13.4	
	CL (L/hr)	145.16	6.86	4.72	
	Q (L/hr)	28.74	2.58	8.97	
	Weight on CL	0.75	---	---	
	Weight on V	1	---	---	
RANDOM EFFECT		**Estimate**	**Std. Error**	**RSE%**	**Shrinkage%**
	BSV_{CL}	0.011	0.008	7.23	4.32
RESIDUAL UNEXPLAINED VARIABILITY		**Estimate**	**Std. Error**	**CV%**	
	Proportional error (%)	18.18	1.65	9.08	

PK parameter values estimated by pooling together all data. V: typical value of volume of distribution from central compartment; V2: typical value of volume of distribution from peripheral compartment; CL: typical value of clearance from central compartment; Q: typical value of intercompartment flow; BSV: between-subject variability; Stderr: standard error; CV%: coefficient of variation; RSE%: relative standard error.

Table 2.3.3-5 Individualized PK Parameter Estimates from a Population Approach

ID	Weight (kg)	ηCL	V (L)	V2 (L)	CL (L/hr)	Q (L/hr)
3G	16	0.08	9.70	16.15	139.70	28.74
6J	16	-0.16	9.70		109.31	
6J	15.7		9.52		107.77	
7D	17	0.13	10.31		153.71	
7D	19.2		11.64		168.40	
7D	20.2		12.25		174.93	
8N	21.3	-0.03	12.92		154.50	
8N	20.8		12.61		151.77	
8N	21.4		12.98		155.04	
9B	24.5	-0.02	14.86		173.63	

Individualized PK parameter values from population PK analysis. Weight on CL and V were included in the final model and between-subject variability was estimated on CL. V2 and Q are population typical values. ηCL: between-subject variability on clearance; V: volume of distribution from central compartment; V2: volume of distribution from peripheral compartment; CL: clearance from central compartment; Q: intercompartment flow

To determine a target plasma ALLO concentration range, I evaluated data from a TETS-induced benzodiazepine-refractory SE mouse model (Zolkowska, Wu, and Rogawski 2018). In this model, ALLO given intramuscularly successfully terminated SE in 92% of animals. The range of maximum plasma ALLO concentrations was between 400-900 ng/mL during the time of SE termination (Zolkowska, Wu, and Rogawski 2018). For those reasons, target plasma ALLO concentrations was determined to be 500-1000 ng/mL. Based on my simulations (Figure 2.3.3-6), an IV infusion of *at least 2 mg/kg* would be necessary in order to attain target plasma ALLO concentrations associated with seizure termination rapidly (i.e. within 2 minutes) and remain above this concentration for approximately 10 minutes.

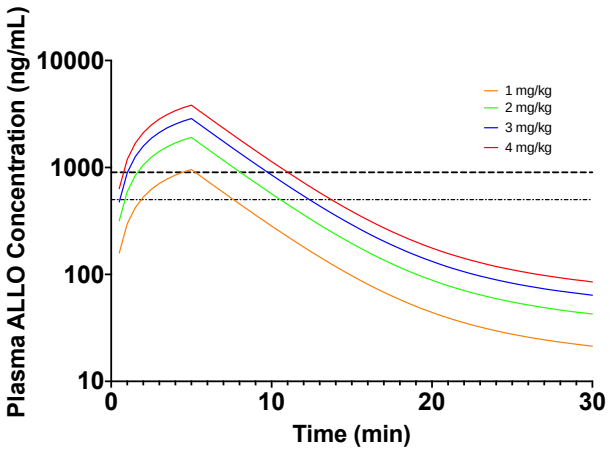

Figure 2.3.3-6 Simulation of Plasma ALLO Concentration-Time Profiles Following IV Dosing. Dashed line: highest peak plasma concentration (900 ng/mL) associated with seizure termination in TETS SE mouse model; Dot-dashed line: lowest peak plasma concentration (400 ng/mL) associated with seizure termination in TETS SE mouse model.

2.3.3.4 Safety and Tolerability

As shown in Table 2.3.3-6, IV ALLO 1-3 mg/kg infused over 5 minutes was shown to be safe and tolerable in dogs. There was a dose-dependent increase in ataxia and sedation. At 4 mg/kg IV, dogs were immobile and briefly unarousable

Table 2.3.3-6 Behavioral Response Following IV Administration

Dose (mg/kg)	n	C_{max} (ng/mL)	Ataxia	Sedation	Ataxia Onset (min post-injection)	Ataxia Duration (min)	Sedation Onset (min post-injection)	Sedation Duration (min)
1	3	425-1340	33%	0%	3	6		
2	4	1654-5287	100%	25%	1.5-2	10-16	3.5	10
3	2	2440-4661	100%	50%	1.5-3	13-13.5	3.5	4.5
4	2	3660-5172	100%	100%	1.5	15.5-18.5	4.5-5	5-6

even with pain stimulation for 1-3 minutes with stable vital signs. Ataxia occurred within 1.5-3 minutes following the start of infusion for a duration of 10-18.5 minutes. In healthy dogs, the onset of sedation occurred at 3.5-5 minutes following the start of infusion and lasted up to 6 minutes at doses greater than 2 mg/kg. The dog on chronic PB had greater sedation at 2 mg/kg, which was

associated with higher plasma ALLO concentrations. There were no infusion site reactions observed.

2.3.4 Discussion

Intravenous ALLO is a compound with a short elimination half-life and a moderately large volume of distribution in dogs. Across the doses studied, IV ALLO exhibited linear pharmacokinetics with respect to dose. This information will be useful for instances where a manipulation of ALLO exposure (e.g. doubling the dose to double the exposure, up to 4 mg/kg) may be necessary during clinical trials. This is in agreement Zulresso PK, as it has been shown to exhibit dose-proportional increases in exposure within the dose range of 0.72-6.48 mg/kg/day (FDA CDER 2019).

Despite including an allometric exponent of weight on clearance and volume to explain some of the residual variability, there remains large interindividual variability that I am unable to explain given the small number of animals and experiments in the present study. The inability to estimate the between-subject variability on volume of distribution without high eta shrinkage may be due to the small number of observations in several animals (Xu et al. 2012). It should also be noted that in the animals such as 6J in whom there were as few as three concentration-time data points available, the PK parameter estimates may be biased since the data points were not sampled at optimized time points and because the weight of each data point is much more significant when there are only a few. With a larger sample size and optimized sampling

times, I may be able to estimate the interindividual variability of intercompartmental clearance and volumes of distribution (central and peripheral) in addition to evaluating covariate effects such as sex or PB.

While the simulations suggested a dose of at least 2 mg/kg would be necessary to attain target plasma concentrations, the safety data provided insight into plasma concentrations associated with heavy sedation (i.e. >2440 ng/mL). Ideally, a dose that can attain high enough plasma concentrations without eliciting deep sedation would be desirable. Therefore, a 2 mg/kg dose infused IV over 5 minutes would be an appropriate dose to test for efficacy in treating canine established SE given these criteria. This dose compares to 1.1 mg/kg in humans using the FDA conversion factor to determine a human equivalent dose and is predicted to attain plasma ALLO concentrations above the target concentration of 1000 ng/mL rapidly without causing sedation in most animals.

2.3.5 Conclusions

This study demonstrates IV ALLO exhibits dose-proportional increases in exposure within 1-4 mg/kg. This would be valuable information to know when designing a clinical trial in canine SE in order to easily calculate the appropriate dose to attain specific target plasma concentrations. IV ALLO is safe and tolerable when administered at doses between 1-3 mg/kg. The onset of ataxia and sedation following IV infusion is rapid and transient, both desirable for the termination of and recovery from SE.

2.4 Pharmacokinetics, Safety, and Optimization of an Intramuscular Allopregnanolone Formulation

2.4.1 Introduction

Although IV administration is the best option to rapidly attain high ALLO concentrations in blood and the CNS, this route is limited by the need for trained personnel to establish an IV line and administer the drug. This often results in a delay in treatment. Ideally, the drug should be administered at the onset of SE, which most often occurs in a pre-hospital setting. Parenteral routes offer the advantage of bypassing the difficulty of oral administration in a seizing patient and avoiding first-pass metabolism. Of these alternative routes, IM administration is a route that requires little technical training or the need to remove much clothing (as compared to a rectal administration).

My working hypotheses are 1) IM ALLO will have relatively high bioavailability, 2) IM injection of ALLO is safe in dogs, and 3) formulation concentration and injection volume will affect bioavailability. The specific aim of this study was to characterize the PK and safety of IM ALLO. The primary objectives of this study were to develop a PK model that best fit concentration-time data, estimate the PK parameter values specifically bioavailability at all doses, assess its safety and tolerability, and simulate dosing regimens that can be used to inform dosing recommendations for a clinical study of CSE. The results of this study will be used to advise further development of an IM formulation and dosing recommendations for a clinical study of CSE.

2.4.2 Methods

2.4.2.1 Study Animals and Safety Monitoring

Four dogs with (n=2) and without (n=2) a history of seizures were used. One of the dogs with a history of seizures had recurrent seizures despite being on phenobarbital (PB) maintenance regimen. Approval was obtained through the Institutional Animal Care and Use Committee of the University of Minnesota prior to the initiation of the study. The dogs were housed at the University of Minnesota's Veterinary College. The dog on PB was previously implanted with a device which wirelessly transmits continuous iEEG recordings (Kremen et al. 2018). On study days, each dog participating in the study was removed from their kennel to have a central-line catheter implanted an hour prior to study start. implanted. Drug administration took place in a procedure room away from the dog's kennel. The dogs were fasted prior to and fed no sooner than 2 hours after drug administration.

2.4.2.2 Study Drug

ALLO concentrate was provided by the Rogawski laboratory at the University of California, Davis. The concentrate was prepared using chemically pure, laboratory grade ALLO and shipped frozen to the University of Minnesota. Three formulations were used:

- 6% ALLO in 24% sulfobutyl ether β-cyclodextrin (Dexolve™) in 0.9% sodium chloride solution (normal saline) diluted and undiluted prior to IM administration for a final concentration of 3-6 mg/mL

- 11% ALLO in 40% Dexolve™ in normal saline, not diluted prior to IM administration
- 14% ALLO in 40% Dexolve™ in normal saline, not diluted prior to IM administration

2.4.2.3 Starting Dose Rationale

Assuming 100% IM bioavailability, a starting dose should be one with an acceptable safety profile following IV administration. In healthy dogs without a history of seizures (regardless of two weeks of PB therapy), 2 mg/kg IV ALLO showed no signs of ataxia or sedation. In one dog on chronic PB therapy, 2 mg/kg IV caused ataxia and sedation. Therefore, the starting dose for IM administration was 2 mg/kg.

2.4.2.4 Study Design

PK studies were completed following IM administration in two healthy dogs and two dogs with a history of seizures (one on chronic PB) in a dose escalation manner, however, in addition to varying the doses, I also administered different injection volumes to test my hypothesis that a high formulation concentration (consequently, a lower injection volume) would increase bioavailability. After the first 2 mg/kg IM study, the bioavailability was approximately 50%. In order to

Table 2.4.2-1 Number of Animals per IM Study Dose

Dose (mg/kg)	1	2			6
Formulation (mg/mL)	11	3	6	14	6
Number of Dogs (total)	1	1	1	1	2
Number of Dogs on PB	1	0	0	0	0
Number of Dogs with EEG	1	0	0	0	0

attempt to attain concentrations comparable to the 3 mg/kg IV dose, the following two dogs were dosed at 6 mg/kg. In total, three doses (1-,2- and 6-mg/kg) across four ALLO concentrations (3-, 6-, 11-, and 14-mg/mL) were evaluated. Whole blood samples (~2 to 5 mL) were collected via a catheterized central vein at pre-dose and at 1-, 3-, 5-, 15-, 30-, 45-minutes, 1-, 2-, 4-, and 6-hours post-injection. A washout period of at least 1 week was used.

2.4.2.5 Allopregnanolone Assay

Whole blood was collected in EDTA-containing purple-top vacutainer tubes and centrifuged for plasma separation as described in Chapter 2.3.2.5.

2.4.2.6 Pharmacokinetic Analysis

ALLO concentration–time data were analyzed using non-compartmental analysis (NCA) (Phoenix 64, Build 8.0.0.3176, Certara L.P., Princeton, NJ, USA). Data was uniformly weighted as described in Section 2.3.2.6.1, with the addition of determining the maximum concentration (C_{max}), time at which maximum concentration is achieved (t_{max}), and bioavailability (F). Due to the large interindividual variability, IM bioavailability (F%) was calculated using equation *(4)*, where the individual's IV and IM $AUC_{0\text{-last}}$'s were compared. $AUC_{0\text{-last}}$ estimates were used rather than $AUC_{0\text{-INF}}$ due to the large percent extrapolated AUC in 3G (>30%).

Equation:

$$F\,(\%) = \left(\frac{AUC_{IM}}{AUC_{IV}}\right)\ x\ \left(\frac{Dose_{IV}}{Dose_{IM}}\right)\ x\ 100 \quad (4)$$

PK parameters were also determined using individual compartmental modeling (Phoenix Non-Linear Mixed Effects 8.0). Due to the exploratory and "n of 1" nature of these IM studies, a population approach was not used. First-order conditional estimation extended least squares method was used as described in Section 2.3.2.6.2.

Individual PK parameter estimates from one dog in whom multiple doses (2- and 6-mg/kg) and formulations (3- and 6-mg/mL) were studied were used to simulate plasma ALLO concentration-time profiles of a "typical" dog given 1-8 mg/kg of IM ALLO using a formulation concentration of 6 mg/mL.

2.4.2.7 Deconvolution Analysis

In addition to calculating bioavailability via the standard method of dividing drug exposure (AUC, determined by NCA) following two routes of administration, bioavailability can be calculated as the cumulative fraction absorbed using deconvolution. Deconvolution is based on the assumptions of the linear superposition principle (Rowland and Tozer 2011). Deconvolution can also be used to determine drug input rate into systemic circulation from varying drug formulations (e.g. oral immediate-release vs extended-release tablets, dermal patches, etc.).

Deconvolution is based on the idea that the concentration-time data of an extravascular dose is a convolution of the input rate and drug disposition (Rowland and Tozer 2011). Therefore, with knowledge of two of these

components, one could calculate the third component. For example, once concentration-time data following IV and IM administration are known, the input rate into the compartment where concentration measurements are drawn (i.e. the central compartment, plasma) and the cumulative amount absorbed can be calculated.

Phoenix WinNonlin deconvolution function was performed to determine the input rate following IM administration using individual PK parameters following IV administration as the exponential terms of the unit impulse function (Table 2.3.3-3). Concentration-time data from the IM administration were used as the response function.

2.4.2.8 Safety and Behavioral Response Evaluations

A modified Glasgow Coma Scale (mGCS) was used to quantitate degree of sedation pre-dose and at scheduled blood sampling times as described in Chapter 2.3.2.7, with the addition of inspecting the injection site for swelling, tenderness, and redness. A muscle biopsy was performed in the dog who received the formulation of highest concentration to assess for evidence of inflammation and/or tissue damage (3G, 14 mg/mL).

2.4.3 Results

The demographics of the dogs are listed in Table 2.4.3-1.

Table 2.4.3-1 Animal Demographics

ID	*Age (years)*	*Gender*	*Weight (kg)*	*Breed*	*Seizure Type*	*Seizure Frequency*	*Co-medications*
3	9	Male, neutered	16	Beagle	History of one witnessed seizure	None	None
6	13	Male, neutered	16	Keeshound Mix	Focal, with generalized seizures	Focal cluster seizures every 14-60 days. With secondarily generalized seizures every 1-2 months.	PB
7	1	Female, intact	17	Coonhound Mix	(healthy)	None	None
8	1	Female, intact	22	Coonhound Mix	(healthy)	None	None

2.4.3.1 Non-compartmental Analysis

The concentration-time profiles following IM administration are shown in Figure 2.4.3-1. Pharmacokinetic parameters estimated by NCA are summarized in Table 2.4.3-2. Apparent clearance ranged from 6.0-12.2 L/hr/kg and apparent volume of distribution ranged from 4.9-44.7 L/kg. Dose-normalized C_{max} following

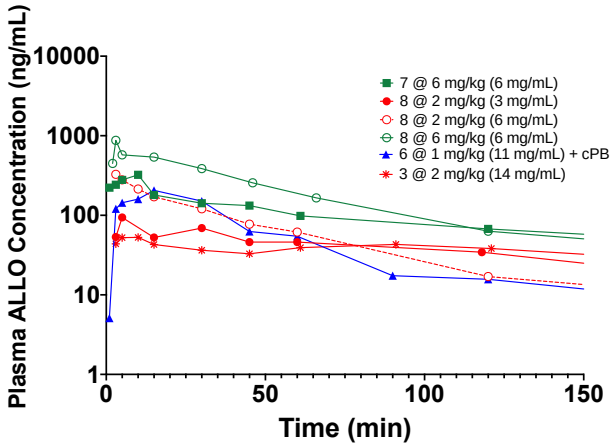

Figure 2.4.3-1 Plasma ALLO Concentration-Time Profile Following IM Administration. cPB: Chronic PB. Blue: 1 mg/kg dose; Red: 2 mg/kg dose; Green: 6 mg/kg dose

Table 2.4.3-2 Non-compartmental PK Parameter Estimates

Form. (mg/mL)	*Dose (mg/kg)*	*n*	*t_{max} (min)*	*C_{max} (ng/mL)*	*λ_z (hr^{-1})*	*$t_{1/2}$ (hr)*	*CL/F (L/hr/kg)*	*V/F (L/kg)*	*$AUC_{0-\infty}$/Dose (ng/mL* hr/(ng/kg)) x 10^{-5}*	*F (%)*
3	2	1	5	94	0.50	1.4	11.4	22.9	8.78	51
6	2	1	3	328	1.23	0.56	10.1	8.2	9.86	62
6	6	2	3-10	323-877	0.27-0.60	1.2-2.5	9.5-12.2	15.9-44.7	8.19-10.5	56-65
11	1	1*	15	205	1.25	0.56	6.0	4.8	16.6	96
14	2	1	10	53	0.32	2.13	5.1	15.8	19.5	112

Estimates are presented as a range. (*) denotes dog on chronic PB. t_{max}: time at maximum concentration; C_{max}: maximum observed plasma concentration; λ_z: terminal phase slope; $t_{1/2}$: elimination half-life; CL: clearance; Vd: volume of distribution; AUC_{∞}: area under the concentration-time curve from time 0 extrapolated to infinity; F: bioavailability

IM administration ranged between 4.7-20.5 x 10^{-5} ng/mL at 3-10 minutes, with a terminal phase half-life between 34-152 minutes. The IM bioavailability calculated by the NCA method was estimated to range between 51-112%. There was a

formulation concentration-related increase in bioavailability with the 14 mg/mL formulation resulting in greater than 100% bioavailability (Figure 2.4.3-2).

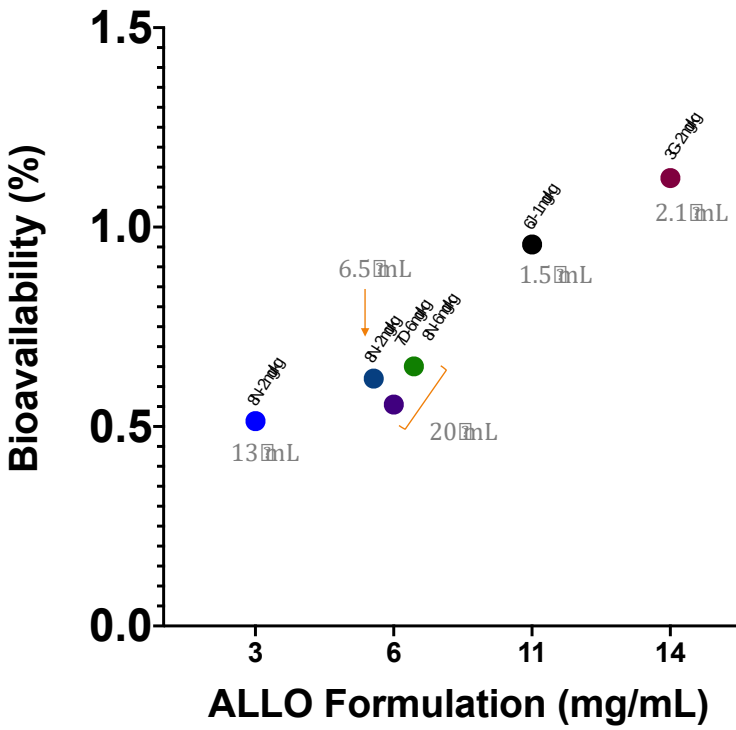

Figure 2.4.3-2 Bioavailability by Formulation Concentration

2.4.3.2 Individual Compartmental PK Analysis Following IM Administration

A two-compartment model with first-order elimination with a proportional error model best fit the ALLO concentrations following IM administration (goodness of fit plots included in Figure 2.4.3-3). Individual parameter estimates are

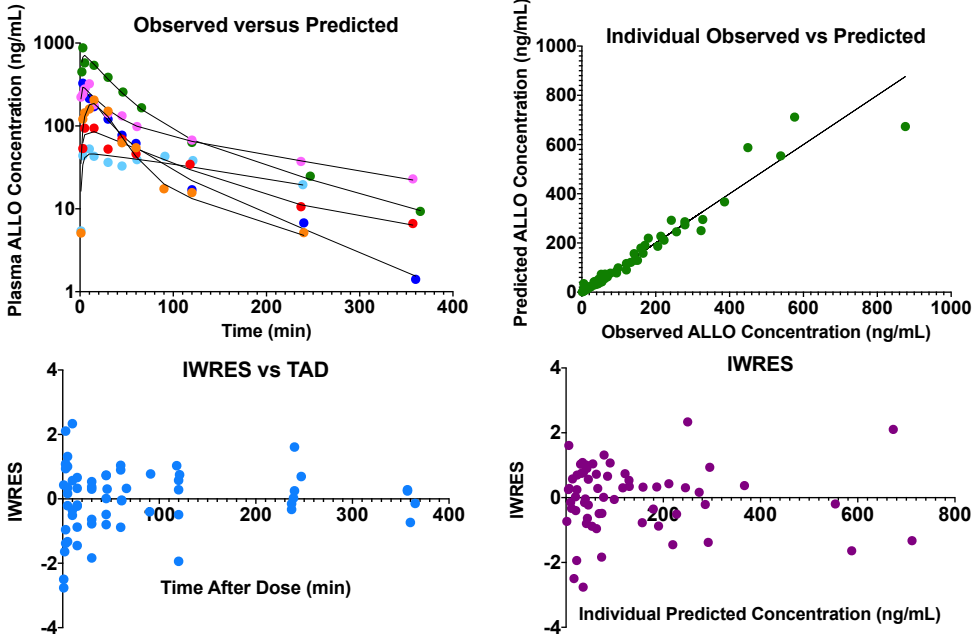

Figure 2.4.3-3 Goodness of fit plots for a two-compartment model. IWRES: individual weighted residual; TAD: time after dose; IPRED: individual predicted concentration

Table 2.4.3-3 Individual Compartmental PK Parameter Estimates

ID	*Dose (mg/kg)*	*Form. (mg/mL)*	*k_a (1/hr)*	*CL/F (L/hr)*	*Q/F (L/hr)*	*V/F (L)*	*V2/F (L)*	*Proportional Error (%)*
8N	2	3	0.68 (52%)	---	312.0 (2286%)	14.8 (67%)	940.1 (5061%)	15.3 (28%)
8N	2	6	2.87 (28%)	202.9 (---)	161.6 (---)	0.8 (---)	137.4 (---)	11.6 (22%)
8N	6	6	2.06 (19%)	193.3 (5%)	115.0 (32%)	7.7 (46%)	164.3 (13%)	14.3 (23%)
7D	6	6	2.60 (33%)	234.0 (6%)	671.9 (35%)	14.0 (48%)	642.2 (11%)	12.4 (22%)
6J	1	11	3.88 (146%)	100.7 (11%)	44.5 (36%)	28.0 (162%)	60.0 (53%)	30.9 (23%)
3G	2	14	0.39 (281%)	160.1 (32%)	91.6 (754%)	10.1 (282%)	151.1 (725%)	26.8 (23%)

Form.: ALLO formulation; k_a: absorption rate constant; CL/F: apparent clearance from central compartment; Q/F: apparent intercompartment clearance; V/F: apparent volume of distribution from central compartment; V2/F: apparent volume of distribution from peripheral compartment.

summarized in Table 2.4.3-3. With the exception of the 14 mg/mL formulation, the rate of absorption increased with formulation concentration.

2.4.3.3 IM Absorption Rate and Bioavailability by Deconvolution Analysis

Across all experiments, the calculated IM absorption rate is highest in the initial 10-15 minutes following injection (Figure 2.4.3-4). Excluding the 14 mg/mL

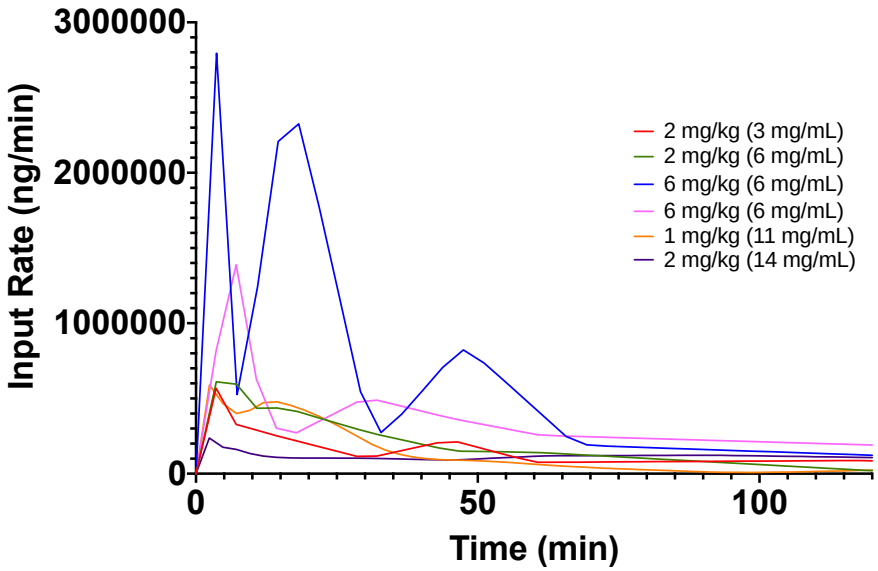

Figure 2.4.3-4 IM ALLO Absorption Rate Over Time

formulation, the higher absorption rates occurred at the combination of higher dose and higher concentration.

Cumulative fraction absorbed over time is illustrated in Figure 2.4.3-5. The absorption follows a first-order rate, except in one dog (3G) administered 2 mg/kg at the highest formulation concentration tested (14 mg/mL). Absorption following this IM injection appears to follow a zero-order absorption rate, which raises concern for the possibility of agglomeration, crystallization, and/or precipitation of drug. However, the tissue biopsy completed in this animal yielded inconclusive

results (no muscle tissue in sample). In one animal (8N) in whom multiple formulations and doses were studied, there appeared to be a formulation concentration-dependent increase in the cumulative fraction absorbed, which supports the observation of increasing absorption rate constant with higher formulation concentration noted in the individual PK analysis.

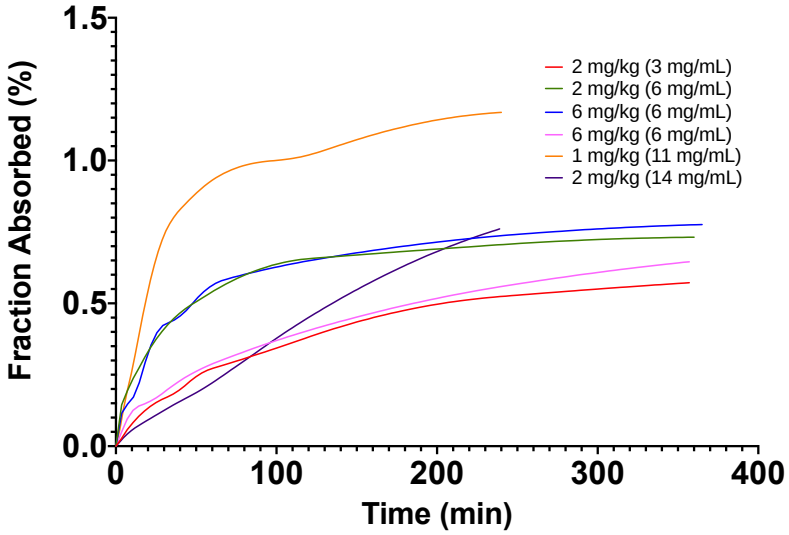

Figure 2.4.3-5 IM ALLO Fraction Absorbed Over Time

The bioavailability estimated by the NCA approach was compared to the cumulative fraction absorbed estimated by deconvolution. The results are shown in Figure 2.4.3-6 and Table 2.4.3-4.

Table 2.4.3-4 Bioavailability Calculated by NCA and Deconvolution Methods

Animal	8N	8N	8N	7D	6J	3G
Dose (mg/kg)	2	2	6	6	1	2
Formulation (mg/mL)	3	6	6	6	11	14
Calculation Method						
NCA	0.51	0.62	0.65	0.56	0.96	1.12
Deconvolution	0.57	0.73	0.78	0.65	1.17	0.76

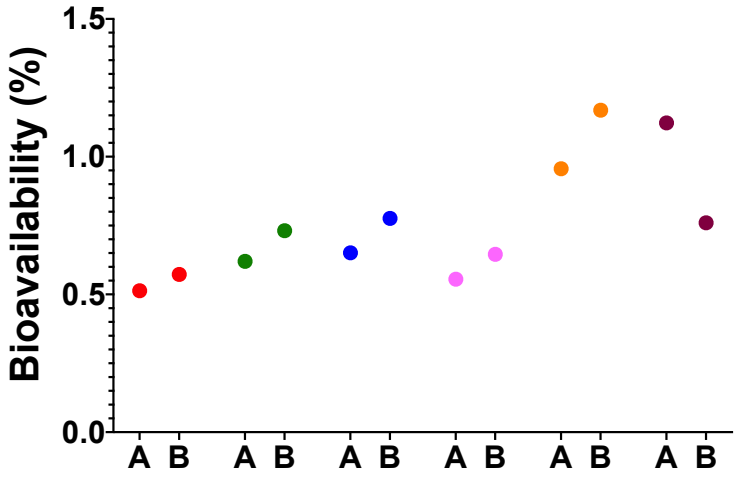

Figure 2.4.3-6 Bioavailability Estimated by NCA vs Deconvolution Methods. A: NCA; B: deconvolution

Based on our simulations of a “typical dog” using a 6 mg/mL ALLO formulation (Table 2.4.3-5), an IM dose of 8 mg/kg would be required to attain the target plasma ALLO concentration of 900 ng/mL and maintain above this concentration for about 5 minutes.

Table 2.4.3-5 PK Parameter Estimates Used for IM Simulations

ka (min^{-1})	CL/F (L/hr)	Q (L/hr)	V/F (L)	V2/F (L)
0.034	193.31	114.96	7.65	164.32

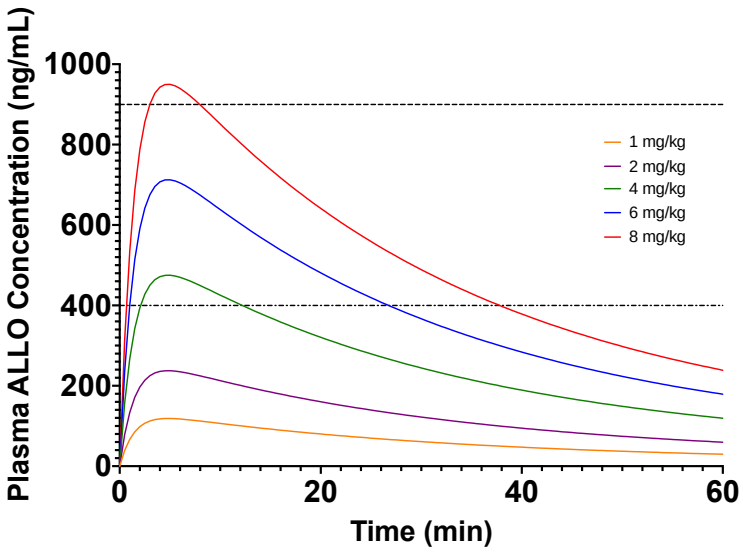

Figure 2.4.3-7 Simulation of Plasma ALLO Concentration-Time Profiles Following IM Administration. Dashed line: highest peak plasma concentration (900 ng/mL) associated with seizure termination in TETS SE mouse model; Dot-dashed line: lowest peak plasma concentration (400 ng/mL) associated with seizure termination in TETS SE mouse model.

2.4.3.4 Safety and Tolerability

The onset of ataxia without sedation occurred 3-5 minutes following the 6 mg/kg dose and lasted for 17-19 minutes (Table 2.4.3-5). Pain associated with injection volume was observed only at the 6 mg/kg IM dose, and is likely associated with the large injection volume (~20 mL).

Table 2.4.3-6 Behavioral Response Following IM Administration

Dose (mg/kg)	n	C_{max} (ng/mL)	Ataxia	Sedation	Ataxia Onset (min post injection)	Ataxia Duration (min)
1	1	205	0%	0%		
2	3	53-328	0%	0%		
6	2	322-876	100%	0%	3-5	17-19

2.4.4 Discussion

The development of IM ALLO for treatment of SE is limited by the slow absorption rate of ALLO from the IM injection site. When comparing the concentration-time profiles following IV and IM administration (Figures 2.3.3-1

and 2.4.3-1), one can notice the loss of the biexponential decay in the IM concentration-time profiles. This apparent monoexponential decay in plasma ALLO concentration is likely due to an absorption rate constant much slower than the elimination rate constant or “flip-flop” kinetics. This is also seen by the difference in λ_z estimated following IV (Chapter 2.3.3.1) and IM dosing (ranges: 1.2-8.1 versus 0.27-1.23, respectively). The terminal rate constant has become limited by the slower absorption rate constant (i.e. the body can only eliminate drug as fast as it is being absorbed). The longer half-life with IM dosing may provide an advantage of maintaining plasma ALLO concentrations above a specific concentration for a longer duration. Due to the exploratory nature of these experiments (“n of 1”), there is large variability that I am unable to explain. Simulations suggest an 8 mg/kg IM dose would be necessary to achieve target plasma concentration window of 500-1000 ng/mL using a 6 mg/mL formulation, however this would result in a large injection volume (~27 mL for a 20 kg dog) which would limit the absorption rate and cause pain upon injection similar in severity to what was observed at the 6 mg/kg dose.

By evaluating the PK across different doses and formulation strengths, I found that a higher formulation concentration (and most importantly, a lower injection volume) was associated with a faster absorption rate. It has been noted that injection volume does have an inverse relationship with absorption rate for IM injections regardless of the water solubility of the drug (Pfeffer and Van Harken 1981; Hirano, Ichihashi, and Yamada 1981). Other factors that may affect the absorption rate constant include the particle size, cohesiveness of the

dissolved particles, initial injection concentration, injection speed and pressure, and the physiological state of the injection site (Hirano, Ichihashi, and Yamada 1981). I also found that at the highest formulation concentration of 14 mg/mL, the estimated absorption rate constant was not as high as I expected based on the formulation-absorption trend observed at previous doses. This suggests that 14 mg/mL may be too high of an initial injection concentration and has negatively affected the absorption rate. It is tempting to conclude that the ideal IM formulation concentration must be between 11- and 14 mg/mL. However, one caveat to consider is that the 11- and 14-mg/mL formulations were studied in one dog each. It is difficult to determine whether these changes in absorption rate are due to formulation alone or the individual animal. A crossover study would be necessary to answer this question.

The results of the deconvolution analysis were comparable to those of the NCA approach. The advantage of performing a deconvolution analysis is the ability to calculate the absorption profile of each IM injection, which may reveal complex absorption patterns. Although the individual PK analysis was able to estimate a first-order absorption rate, it is unable to capture any absorptive activity following the initial peak. In the present study, there was large interindividual and inter-formulation variability in absorption profiles. However, due to the small number of animals and number of IM studies per animal, it is difficult to attribute the large variability to drug disposition kinetics alone. A second peak was seen in all dogs (except for one occasion at 2 mg/kg [6 mg/mL]) at 20-45 minutes post-injection, and in one dog, a third peak was

observed at 50 minutes post-injection. These secondary peaks in absorption rate suggest a delayed absorption, which may be explained by increased blood flow to the injection site (e.g. walking, playing, or receiving pets from veterinary technicians) and/or a depot effect. In addition, small differences in the estimated bioavailability may be due to the sensitivity of the deconvolution method to errors in the unit impulse function (based on exponential terms provided by individual PK parameter values following IV administration).

2.4.5 Conclusions

This study demonstrates that ALLO is safe and tolerable when administered IM at doses up to 2 mg/kg and at injection volumes less than 10 mL per injection site. Following the 6 mg/kg dose, the onset of ataxia occurred within 5 minutes of IM injection and lasted for almost 20 minutes, which suggests relatively quick brain penetration and moderate residence time. However, at the 6 mg/mL formulation, my simulations suggest an 8 mg/kg IM dose would be necessary to achieve target plasma concentration range of 500-1000 ng/mL, which would result in an injection volume (~27 mL in a 20 kg dog) large enough to limit the absorption rate and cause pain with injection. Therefore, based on absorption rate limitations, the development of IM ALLO would require alternative approaches to be feasible.

2.5 Pharmacodynamics of Intravenous and Intramuscular Allopregnanolone in Dog

2.5.1 Introduction

Allopregnanolone has been shown to effectively terminate seizures in several acute seizure and SE rodent models (Rogawski et al. 2013; Kokate et al. 1996; Cheryl A Frye and Scalise 2000; Zolkowska, Wu, and Rogawski 2018). It has also been reported to affect electroencephalograph parameters in a fashion similar to benzodiazepines (BZDs) in rats, causing increases in higher-frequency (beta) activity (Lancel et al. 1997; Slawecki, Purdy, and Ehlers 2005). Based on behavioral observation of our study animals alone, ALLO appears to have a fast onset of action, inducing ataxia as early as 90 seconds and sedation just 3.5 minutes following the start of IV infusion. However, it remains unknown whether ALLO can affect electrical brain activity in a manner that could stop and/or prevent further seizures in dogs.

For these studies, I hypothesized that 1) ALLO has a quick onset of effect on intracranial electroencephalograph (iEEG) activity in dogs, and 2) based on its potentiation of $GABA_A$ currents, its effect on iEEG is similar to that of benzodiazepines (i.e. increase in beta activity) (Mandema, Kuck, and Danhof 1992; Mandema and Danhof 1992). Alternative hypotheses include 1) ALLO has a moderate or slow onset of effect on iEEG, and 2) its effect on iEEG can be differentiated from those due to benzodiazepine administration. The specific aim of this study is to develop a PK-PD model relating plasma ALLO concentrations to iEEG response in one dog. The primary objectives of this study were to

estimate the concentration that produces 50% of the maximum response (EC_{50}) and the effect compartment rate constant (k_{e0}) of IV and IM ALLO. The results of this study will be used to inform the target therapeutic concentration and dosing recommendations for a clinical study in CSE.

2.5.2 Methods

2.5.2.1 Study Design

One dog with a history of seizures was used. Despite being on a PB antiseizure maintenance regimen, he occasionally had breakthrough seizures. Approval was obtained through the Institutional Animal Care and Use Committee of the University of Minnesota prior to the initiation of the study. The dog was housed at the University of Minnesota's Veterinary College. This dog was previously implanted with a device which wirelessly transmits continuous iEEG recordings (Kremen et al. 2018). On study days, the dog was removed from his kennel to have a central-line catheter emplaced an hour prior to study start. On days where IV ALLO was administered, a peripheral-line catheter was also emplaced. Drug administration took place in a procedure room away from the dog's kennel. The dogs were fasted prior to and fed no sooner than 2 hours after drug administration.

iEEG data were collected in the dog during two IV studies (1-2 mg/kg) and one IM study (1 mg/kg). Each collection period started the day before and finished the day after IV injection to determine baseline data. iEEG data were recorded continuously at a sampling rate of 250 Hz.

2.5.2.2 Intracranial Electroencephalographic (iEEG) Analysis

iEEG data were analyzed using custom algorithms (Matlab, version 2018b) for characterizing iEEG power in bands of interest and its temporal dynamics (Delta (1 -3 Hz), Theta (>3 – 7 Hz), Alpha (>7 - 12 Hz), Beta (>12 – 25 Hz), High Beta (>20 -25 Hz)) as described previously (Kremen et al. 2017). Absolute and relative power densities across frequency bands were used as the pharmacodynamic response. Absolute power density refers to the absolute power (voltage) of the patient's iEGG, while relative power density refers to the percentage of power a frequency band (such as beta) compared to the sum of all frequency bands (delta, theta, alpha, and beta).

2.5.2.3 Pharmacokinetic-Pharmacodynamic (PK-PD) Modeling

Individualized PK parameters were estimated using a population approach as described in Section 2.3.2.6.2 (Table 2.3.3-5), fixed, then indirect-link Emax/Imax PD models were explored. An indirect-link was used due to the hysteresis that was observed. The best fit model was determined using visual inspection and precision of model parameters.

2.5.3 Results

2.5.3.1 Intracranial Electroencephalographic Analysis

2.5.3.1.1 Absolute power density

Across all iEEG frequency bands, absolute power density increased during and transiently after IV ALLO (Figure 2.5.3-1). The beta frequency band (purple) appears to occur at the lowest plasma ALLO concentrations. As the dose increased, the amplitude and duration of these iEEG changes also increased. Following IM injection, plasma ALLO concentrations did not reach high enough concentrations to produce iEEG changes comparable to the IV infusion at the same dose and changes were more spread out over time. However, changes in absolute power densities at all doses were statistically significantly different

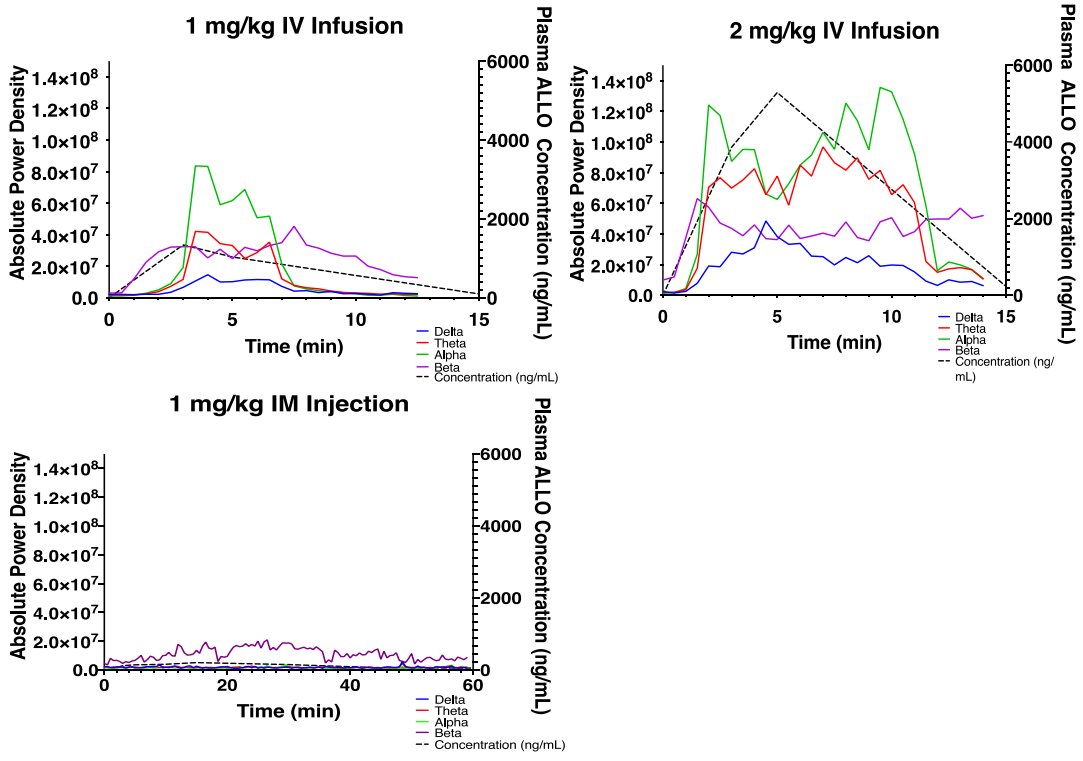

Figure 2.5.3-1 Absolute Power Density and Plasma ALLO Concentration-Time Profiles Following IV and IM ALLO Administration in One Dog on Chronic PB.
Colored solid lines: iEEG, separated into four frequency bands; dashed black line: plasma ALLO concentration

compared to pre-dose and non-study day baselines irrespective of route of administration.

2.5.3.1.2 Relative power density

High beta frequency band (20-25 Hz) relative power density decreased, while alpha frequency band (7-12 Hz) relative power density increased during and transiently after IV infusion (Figure 2.5.3-2). These changes occurred for a longer duration but not to a larger extent at the higher dose. As with absolute power density, relative power density changes following IM injection were not as drastic as changes seen following IV infusion.

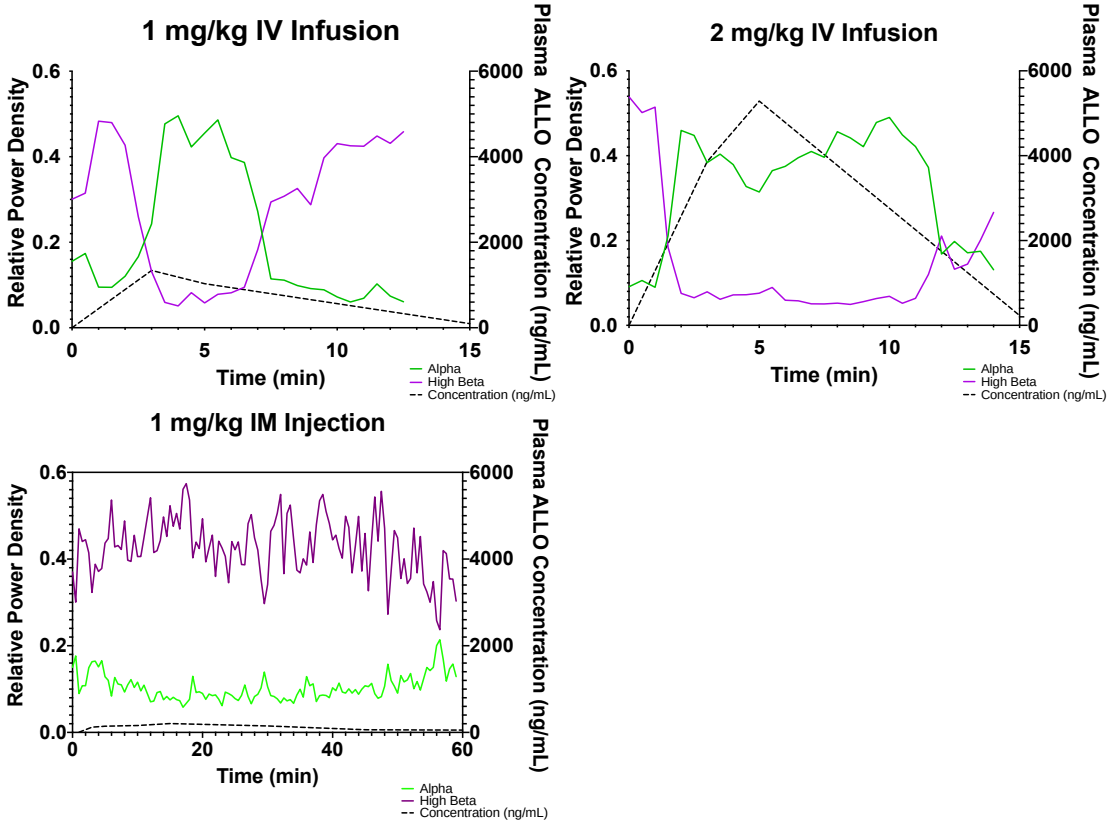

Figure 2.5.3-2 Relative Power Density and Plasma ALLO Concentration-Time Profiles Following IV and IM ALLO Administration in One Dog on Chronic PB.
Purple solid line: Relative beta power density; green solid line: relative alpha power density; dashed black line: plasma ALLO concentration

2.5.3.2 Pharmacokinetic-Pharmacodynamic Analysis

For all frequency bands, changes in absolute power density in response to plasma ALLO concentrations were best described by an indirect-link sigmoidal Emax model (Table 2.5.3-1 and Figure 2.5.3-3). There was an overall increase in brain activity following ALLO administration, especially via the IV route. Following IV administration, the delay equilibrium half-life ranged from 8-32 seconds, suggesting the time required to reach the full response in the brain is less than 3 minutes. The delay equilibration rate constant could not be calculated with precision for most frequency bands following IM injection, likely due to a lack of significant change in absolute power density. The beta frequency band (comprised of the low and high beta frequency range) had the lowest EC_{50} (270

Table 2.5.3-1 PKPD Parameter Estimates (iEEG Absolute Power Density)

Frequency Band	***k_{e0} (1/min)***		***EC_{50} (ng/mL)***		***E_0 (10^6)***		***E_{max} (10^6)***	
Route	***IV***		***IV***		***IV***		***IV***	
Dose	***1***	***2***	***1***	***2***	***1***	***2***	***1***	***2***
Delta (1-3 Hz)	2.93 (18.2)	1.42 (24.4)	652 (2.0)	700 (20.3)	2.6 (9.9)	0.1 (>500)	9.1 (6.6)	37.1 (23.2)
Theta (3-7 Hz)	3.75 (29.6)	1.32 (27.8)	666 (2.6)	538 (6.1)	2.9 (38.2)	9.4 (36.4)	32.3 (8.6)	68.5 (6.9)
Alpha (7-12 Hz)	5.05 (35.4)	1.32 (13.7)	681 (2.3)	499 (4.1)	2.3 (86.8)	8.8 (81.0)	67.7 (7.8)	92.3 (9.2)
Beta (12-25 Hz)	2.00 (22.4)	1.60 (26.3)	276 (6.7)	270 (25.9)	4.5 (55.3)	13.0 (39.6)	27.7 (10.7)	32.5 (16.5)

Values are presented with coefficient of variation (%). k_{e0}: delay equilibrium rate constant; EC_{50}: concentration in the effect compartment required to elicit half of the maximum response; E_0: baseline response; E_{max}: maximum response

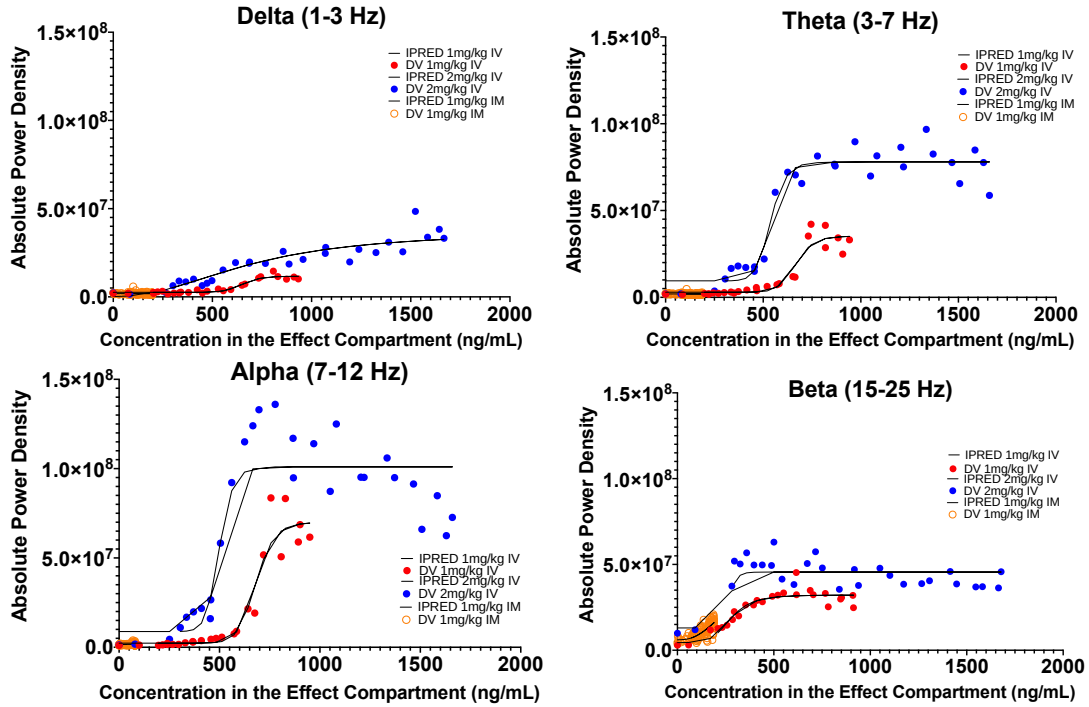

Figure 2.5.3-3 iEEG (absolute power density) effect-concentration profiles following 1-2 mg/kg infused IV over 5 minutes (red and blue, respectively) and 1 mg/kg injected IM as a bolus (orange) in 1 dog on cPB.
Effect compartment concentrations are predicted using an indirect-link between PK and sigmoidal Emax PD models. Colored circles are observed data; solid lines represent predicted data. IPRED: predicted effect; DV: observed effect.

ng/mL) compared to the other frequency bands, suggesting it could be a surrogate marker useful in clinical studies as a signal for CNS penetration. This would approximate 400 ng/mL in the plasma.

Concentration-iEEG data using relative power densities were also examined. Only alpha and high beta frequency bands had consistent changes in relative power density with an increasing IV dose. In contrast to changes observed in absolute power density, relative power density of high beta decreased after administering ALLO, while relative power density of the alpha band increased. In other words, relative to all the increasing brain activity, the input higher beta frequency band is decreasing in response to ALLO. The relative power density changes and concentration-time data for high beta and alpha frequency bands were best fit by an indirect-link sigmoidal Imax and Emax models, respectively (Table 2.5.3-2 and Figure 2.5.3-4). Similar to absolute

Table 2.5.3-2 PKPD Parameter Estimates (iEEG Relative Power Density)

Frequency Band	***k_{e0} (1/min)***		***EC_{50} (ng/mL)***		***E_0 (10^6)***		***E_{max} /I_{max} (10^6)***	
Route	***IV***		***IV***		***IV***		***IV***	
Dose	***1***	***2***	***1***	***2***	***1***	***2***	***1***	***2***
Alpha (7-12 Hz)	2.35 (25.0)	1.20 (10.6)	759 (14)	455 (4.2)	0.09 (19.6)	0.10 (25.4)	0.57 (36.6)	0.32 (8.8)
High Beta (20-25 Hz)	2.27 (26.5)	0.92 (5.1)	635 (10)	329 (3.0)	0.43 (4.7)	0.52 (3.3)	0.47 (22.4)	0.45 (4.2)

Values are presented with coefficient of variation (%). k_{e0}: delay equilibrium rate constant; EC_{50}/IC_{50}: concentration in the effect compartment required to elicit half of the maximum (inhibitory) response; E_0: baseline response; E_{max}/I_{max}: maximum (inhibitory) response

power density changes, relative power densities had a larger change and for a longer duration at the higher IV dose, no significant changes following IM dosing, and changes occurred at lower concentrations in the high beta frequency band compared to alpha frequency band. The EC_{50} in the theoretical effect compartment to produce changes in the high beta frequency band is approximately 720 ng/mL in the plasma.

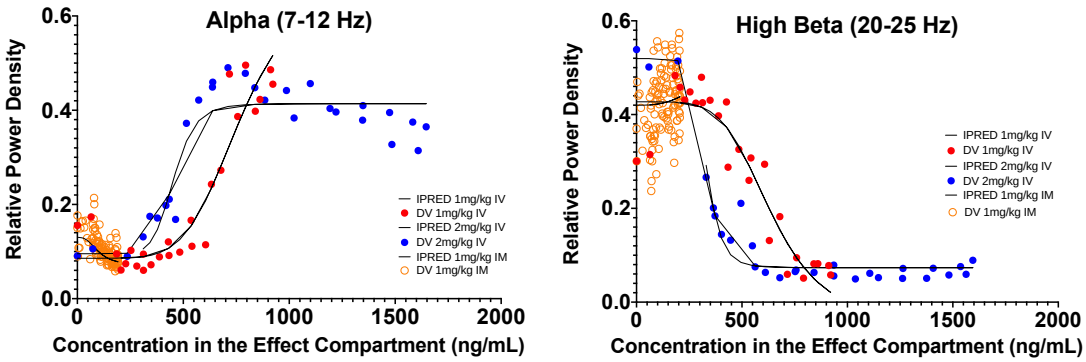

Figure 2.5.3-4 iEEG (relative power density) effect-concentration profiles following 1-2 mg/kg infused IV over 5 minutes (red and blue, respectively) and 1 mg/kg injected IM as a bolus (orange) in 1 dog on cPB.
Effect compartment concentrations are predicted using an indirect-link between PK and sigmoidal Emax/Imax PD models. Colored circles are observed data; solid lines represent predicted data. IPRED: predicted effect; DV: observed effect.

2.5.4 Discussion

Overall, I observed higher absolute power in all bands after administering the drug, suggesting that ALLO increases brain activity. Following increases in IV dose, the extent and duration of iEEG changes also increased. With the higher dose of ALLO, the iEEG changes were greater, the onset of duration was faster, and occurred for a longer period of time. In contrast, following IM dosing, there were statistically significant but minimal changes to iEEG, likely due to the attainment of lower plasma ALLO concentrations. The peak plasma concentration following IM dosing (205 ng/mL) was just below the lowest *plasma* EC_{50} for absolute and relative power density changes estimated from the IV infusions (~400- and 720-ng/mL for beta frequency).

In contrast, increases in absolute and relative power density of the beta frequency band in response to benzodiazepine intervention have been widely cited (Mandema and Danhof 1992; Mandema, Kuck, and Danhof 1992;

Greenblatt et al. 1989; Van Lier et al. 2004; Friedman et al. 1992). In contrast to what I observed following IV ALLO, Van Lier et al reported that diazepam elicits increases in relative power density of high beta (defined as 21-30 Hz) while decreasing alpha (defined as 9-10 Hz) in rats (Van Lier et al. 2004). This suggests that although ALLO and BZDs potentiate $GABA_A$ receptors, they induce differential effects on EEG activity. This may be due to ALLO's activity at synaptic and extrasynaptic $GABA_A$ receptors while BZDs have activity at only synaptic $GABA_A$ receptors.

The frequency band that appears to have highest sensitivity for plasma ALLO concentrations is beta (15-25 Hz). For absolute and relative power density changes, beta frequency changes occur at EC_{50}/IC_{50} lower than other frequency bands. Interestingly, the estimated EC_{50} for beta changes following IV ALLO are comparable to what has been observed following a clinically-relevant IV diazepam dose (0.15 mg/kg) for percent change over baseline beta activity in healthy human volunteers (400 ng/mL vs. 270 ng/mL, respectively) (Greenblatt et al. 1989).

Another PD model that could have been used to describe the iEEG data is an indirect-response model, using either stimulation of the "rate in" or inhibition of the "rate out" (Gabrielsson and Hjorth 2016). However, many of the previous exposure-response models developed with benzodiazepines used E_{max} models (Mandema and Danhof 1992; Mandema, Kuck, and Danhof 1992; Greenblatt et al. 1989; Van Lier et al. 2004; Friedman et al. 1992). Furthermore, using an indirect-response model would require the estimation of more parameters, which

may be difficult given the limited data that I have. Therefore, in order to generate comparable results, especially with respect to the beta frequency band, a sigmoidal E_{max} model was used.

It is important to note that this animal (6J) had higher concentrations at the doses administered compared to the other animals who have been administered the same dose. This should be taken into consideration when designing a clinical trial for canine SE, knowing that many of dogs that present in a veterinary emergency department with canine SE are on PB chronically to maintain seizure control.

Zolkowska's work demonstrated that IM ALLO has great potential to be useful as a first-line treatment for SE, but the current formulations do not meet the ideal criteria for a first-line SE treatment (Zolkowska, Wu, and Rogawski 2018). The largest limitation of the IM route of administration is the inability to attain high enough plasma concentrations to elicit iEEG changes. Strategies to increase plasma concentration using clinically-relevant volumes include but are not limited to: 1) increasing its water solubility by synthesizing a pro-drug or using alternative delivery systems; 2) using different administration strategies like multiple injections of changing the needle gauge and/or length; or 3) optimizing the formulation by using multiple solvents.

2.5.5 Conclusion

The onset of effect of IV ALLO, as seen from iEEG changes and behavioral response, was rapid and transient, both desirable for the termination of and recovery from SE. Only apparent following IV infusion, I observed a dose-related

increase in extent and duration of absolute and relative power densities. Because change in absolute and relative power densities for the beta frequency band occurred at the lowest EC_{50} compared to other frequencies, it may be useful as a surrogate marker of drug brain penetration. Although IM ALLO would be ideal for pre-hospital settings, at the IM formulations studied, plasma ALLO concentrations following IM administration are not high enough to induce changes in iEEG activity comparable to those seen following IV infusion due to low absorption rates.

Compiling all of the PKPD and safety data from this project and what has been reported by my collaborators at UC Davis (Figures 2.5.5-1 and 2.5.5-2), I

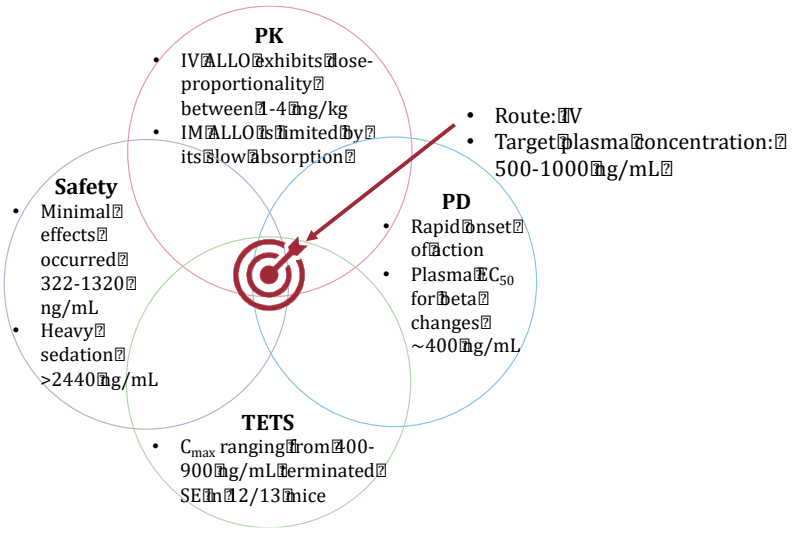

Figure 2.5.5-1 Determination of the Target Plasma ALLO

propose that a 2 mg/kg dose infused over 5 minutes is an appropriate starting dose to test as a first-line agent to treat canine SE, as this dose is predicted to attain the target concentrations (500-1000 ng/mL) without causing heavy sedation. Although a 1 mg/kg dose would also attain plasma concentrations that fall within the range between the EC_{50} and those associated with heavy sedation,

only the 2 mg/kg dose is predicted to attain the peak concentration observed in the TETS SE mouse study (900 ng/mL) and as early as 3 minutes into the start of infusion.

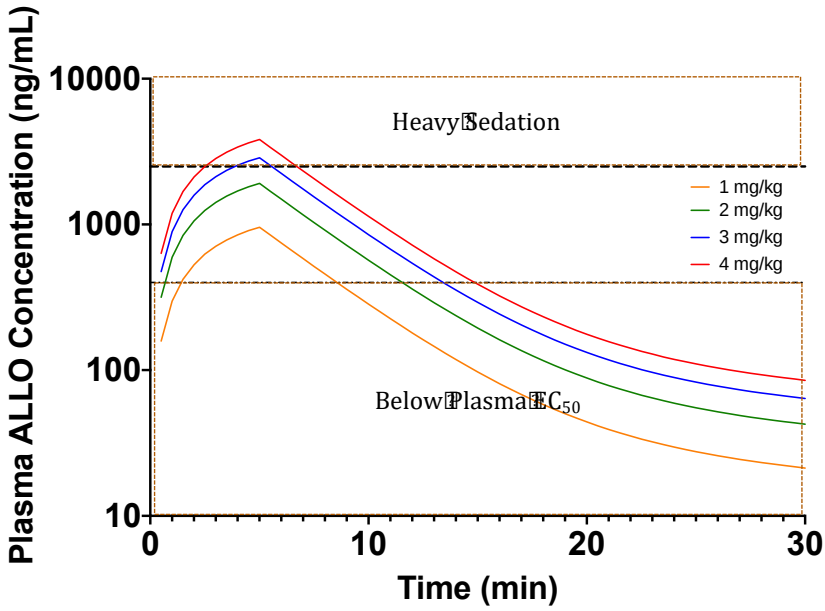

Figure 2.5.5-2 Determination of IV ALLO Dose for a Clinical Trial in Canine SE

2.6 Design of a Safety and Effectiveness Clinical Trial of Intravenous Allopregnanolone for the Treatment of Canine Status Epilepticus

In spite of having established guidelines for the management of convulsive SE, upwards of 40% of patients are clinically non-responsive to benzodiazepines (BZDs) and there remains a critical need to identify a treatment for SE that is efficacious for all patients. Without such a therapeutic agent or regimen, 31-40% SE cases are at risk for untimely death due to prolonged seizure episodes (Hocker et al. 2013; Leitinger et al. 2019). My long-term goal is to find a more effective first-line drug for SE treatment that has the potential of replacing or being used in conjunction with BZDs. IV ALLO is an ideal agent to treat this

emergent condition based on its mechanisms of action and its ability to rapidly diffuse into the CNS.

The development path for potential approval of IV ALLO for the treatment of seizure emergencies requires conducting clinical trials that safety and effectiveness. Because IV ALLO has been approved for another indication, much of the toxicity and safety studies may not be required. A unique pathway to encourage development of IV ALLO for SE in people is first establishing safety and efficacy in canine patients prior to human use. Although this approach would not replace the need for safety and effectiveness trials in people, the results would inform the design of a clinical trial in human SE. The following proposed study have a primary objective of characterizing the safety and effectiveness of ALLO for the treatment of SE in dogs. Evaluating potential therapies for SE in dogs with naturally-occurring epilepsy and SE have the added advantage of developing life-changing treatments for dogs and people.

2.6.1 Study Objective

The primary objective for this study is to demonstrate that the effectiveness and safety of IV ALLO as an agent for early CSE is as effective as IV DZP.

2.6.2 Study Population

Dogs (5-40 kg in body weight) admitted into an emergency/urgent veterinary medical clinic with a clinical diagnosis of convulsive status epilepticus and have received at least one adequate dose of a BZD will be enrolled. CSE is defined

as continuous convulsions lasting >5 min, or 2 or more recurrent convulsions without regaining consciousness between seizures within the last 12 hours and seizures are continuing to occur or likely to reoccur without recovery in between. Canine patients may be excluded from the study for the following reasons: owner not wishing to participate, anoxic cause for status epilepticus, or metabolic cause for status epilepticus (i.e. must have normal blood glucose, calcium, bilirubin, etc. on admission bloodwork).

2.6.3 Study Design

This will be a double-blinded, randomized, multicenter non-inferiority study in canine patients with SE who have failed first-round or second round benzodiazepines study (Figure 5.2.2-1). This study design will be modeled after a prospective, multicenter, randomized parallel-group study comparing the

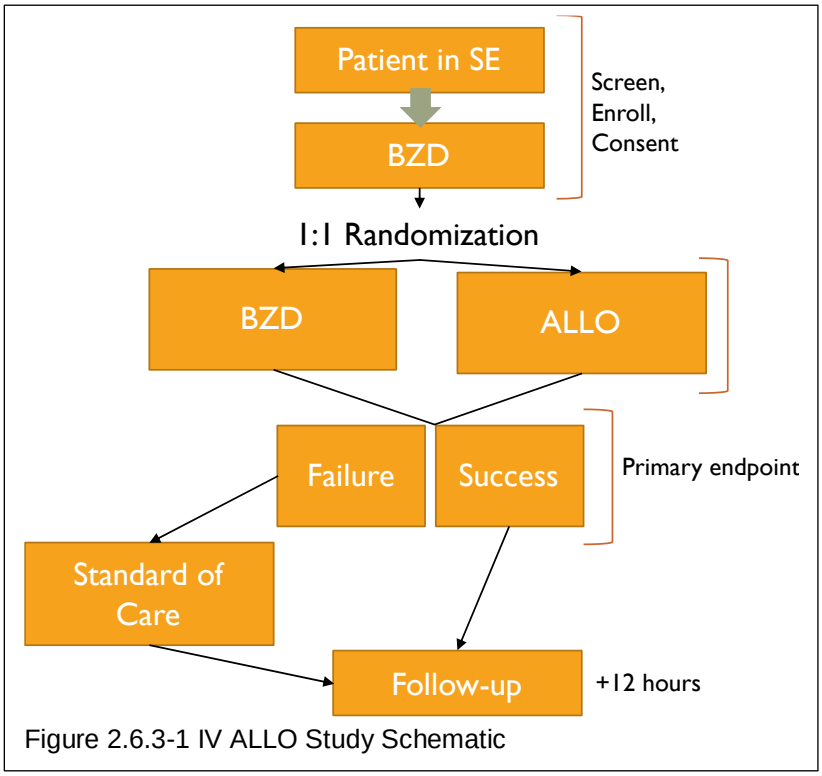

Figure 2.6.3-1 IV ALLO Study Schematic

effectiveness of intranasal midazolam and rectal DZP in terminating CSE within 5 minutes without seizure recurrence in 10 minutes (M. Charalambous et al. 2017). Although I am interested in developing IV ALLO as first-line treatment, it would be unethical to test its effectiveness without giving the canine patient a known effective treatment first.

2.6.4 Endpoints

2.6.4.1 Primary Endpoint

Termination of seizure within 5 minutes of infusion without recurrence of seizures within 10 minutes of dosing. Clinical cessation of SE consists of absence of clinical seizures and improving responsiveness. Absence of apparent seizures will be determined clinically. Responsiveness will be determined by patient's response to verbal command or noxious stimuli.

2.6.4.2 Secondary Endpoints

Secondary endpoints include: Safety/tolerability (degree and duration of ataxia and sedation, heart rate, blood pressure, EKG monitoring, SpO_2 measurement, need for intubation within 60 minutes of start of study drug infusion, seizure cessation at 60 minutes after drug administration, need for rescue therapy, time to next seizure, 12-hour responder rate, discharge status, and presence of subtle CSE via EEG monitoring (when available).

2.6.4.3 Blinding/Unblinding and Randomization

Patients, veterinary emergency department study team members, PIs, and clinical coordinating centers will be blinded to the treatment assignment.

Emergency unblinding may be required if the treating team feels that a patient's care requires knowledge of what study drug was given. Emergency unblinding will not be performed within 60 minutes of the start of study drug infusion with the exception of veterinarian judgment that it is necessary for the safety or care of the patient or because of unanticipated situations accommodated by study procedures. The PK study PIs and laboratory scientists will know the assay results but will remain blinded to response until the completion of the study. Drug concentration data will not be disseminated until the completion of study.

Randomization for this study will be 1:1 between 2 mg/kg IV ALLO and a second-/third-round BZD (0.5 mg/kg IV DZP).

2.6.5 Sample Size

The primary objective of this study is to demonstrate that the number of patients whose seizures terminate within 5 minutes of study drug infusion without seizure recurrence in the IV ALLO group is not inferior to that in the IV DZP group by more than a noninferiority margin of 10%. The null hypothesis of inferiority will be tested using a one-sided test with an 80% power and a significance of 5% (one-sided probability of a type I error of 0.05). The estimate of IV BZD success rate of 55% is from a prospective, double-masked, randomized parallel-group study of IV benzodiazepine (BZD, lorazepam or DZP) + saline infusion and IV BZD + IV fosphenytoin (FOS) for the treatment of CSE (Patterson et al. 2015). In this study, once a seizure occurred in the emergency department, canine patients were given an IV BZD followed immediately by either IV FOS or normal saline,

and success was defined as seizure termination without recurrence within 2 hours. Therefore, the sample size required is 342 per treatment arm (a total of 684 canine patients), which takes into account a 10% inflation to account for loss of follow up, protocol deviation (inclusion/exclusion criteria violation), and/or repeat enrollment of the same subject. This conservative approach of assuming ALLO has an efficacy rate of 55% is to ensure the study has a large enough sample size and sufficient power.

2.6.6 Study Drug

IV ALLO (6 mg/mL in 24% Dexolve, manufactured by University of California Davis) and IV DZP (commercially supplied as 5 mg/mL injectable solution) will be used for this study.

2.6.6.1 Dose Rationale

Based on the population pharmacokinetic model presented in Chapter 2.3.3.3, 2 mg/kg IV ALLO infused over 5 minutes is predicted to attain the target plasma concentration within 2 minutes of the start of infusion and maintain concentrations above this target for approximately 5 minutes without causing heavy sedation. The corresponding dose for DZP is 0.5 mg/kg, representing the standard of care dose for treatment of SE in canines. The concentrations were chosen so that volumes of the two study drugs would be identical based on weight. A Dosing Chart will be provided to enable veterinary clinicians to administer the same volume of either drug based on kg bodyweight. For example, a 5 kg dog will be given 2 mL, while a 40 kg dog will be given 16 mL.

2.6.7 Study Protocol

Dogs arriving at the study centers for emergency treatment of seizures will be considered for enrollment. If they meet the inclusion criteria, and informed consent is obtained, dogs will be entered into the study and assigned a randomized, blinded treatment. The dog may have received 1-2 previous doses of BZDs en route to the study center or upon presentation at the study center. The standard of care for dogs is to initially treat up to 3 times with IV BZDs as they have a shorter half-life in dogs than in humans. If a dog presents to the study veterinary clinic in a continuous seizure, an IV BZD will be given, and then consent for the study offered. A central-line catheter will be placed, and dogs will be admitted to the veterinary ICU for monitoring for 5 hours consisting of: 1) continuous seizure watch; and 2) hourly checks for alertness, vomiting, diarrhea, or salivation; temperature, pulse, and respirations. If the subject recovers fully, the subject will be discharged from the study and $500 of the bill will be covered by the study. If the seizure continues or another seizure occurs within 5 hours or a seizure is continuing after consent is obtained, the canine study patient will receive the randomized treatment: either 0.5 mg/kg DZP IV or 2 mg/kg ALLO IV as a slow bolus over 5 minutes. An aggressive rescue treatment plan, based on the standardized treatment protocol in place at each institution, will be initiated if the canine patient's convulsions do not diminish 10 min after the completion of the infusion or completely stop by 15 min or if motor seizure activity re-occurs within 12 hours. All subjects entered into the randomization phase will be

observed for at least 12 hours after treatment with the study drug and the owners will receive $1500 towards their veterinary care. Appropriate tests will be performed to diagnose the cause of the CSE. If a dog does not survive, a post-mortem exam will be requested.

2.6.8 Data Analysis Plan

All canine patients who receive study medication will be included in the analyses. Continuous demographic and baseline variables such as sex, age, weight, laboratory values (complete blood count, serum chemistry, and bile acids), number of previous BZDs prior to study treatment, and duration of SE and/or number of seizures prior to arrival, will be tested between treatment groups using a two-sample *t*-test; categorical variables such as sex, neuter status, breed (if known), history of epilepsy diagnosis, seizure etiology, and seizure type (if history of epilepsy) will be tested using a Chi-square test. If any of the subgroups are expected to be less than 5 in count, Fisher's exact test will be used.

The primary endpoint will be compared between BZD and ALLO groups using the Chi-square test.

Secondary endpoints including the time to next seizure and duration of ataxia and sedation will be compared between groups using a log rank test. Seizure cessation at 60 minutes after drug administration, need for rescue therapy, need for intubation within 60 minutes of start of study drug infusion, presence of EKG abnormalities, 12-hour responder rate, discharge status, and presence of subtle CSE via EEG monitoring will be compared between groups

using a Chi-square test. Continuous variables such as heart rate, blood pressure, SpO_2 measurement, Ranked/scored endpoints such as degree of ataxia and sedation will be compared between groups using a Mann-Whitney test. In addition, binary secondary endpoints will be compared across clinic sites using the Cochran-Mantel-Haenszel test. If there are statistically significant differences in demographic covariates and/or factors, an exploratory logistic regression analysis can be performed evaluate the influence of these covariates/factors on the probability of treatment outcome.

2.6.9 Challenges and Limitations

One major challenge of this study design is the large sample size required, which may be difficult to recruit for given the low prevalence of CSE (Chapter 1.3.1.1). To overcome this, involving more veterinary medicine centers and extending the study internationally may help with recruitment. However, this approach would also require more time for study set up and staff training. Alternative approaches are discussed in the following section that would require a smaller sample size. Because this study will be done in emergency departments, complete adherence to all aspects of the protocol may be difficult. Deviations can be expected. Collecting blood within the pre-defined collection windows within the emergency department environment may be a challenge. Incomplete data collection will be monitored, and additional training will be implemented where and when needed. The number of patients arriving with CSE varies from time to time, and enrollment may be slower than estimated. To offset this, we can increase the

number of clinic sites. Finally, subjects may be enrolled than later are found to not have met the entry criteria due to the cause of the CSE not having been accurately diagnosed initially. In our study, there will be little need for unblinding, as the study drug administered will not affect the subsequent standard of care, if necessary.

2.6.10 Expected Results and Alternative Approaches

I anticipate that IV ALLO will prove to achieve seizure cessation within 5 min of dosing without seizure recurrence within 60 min as frequently as IV DZP, and that it will be as safe as IV DZP. This will lead to consideration of IV ALLO as a treatment for CSE and will encourage human clinical trials.

If IV ALLO does not exhibit a noninferior effect compared with IV DZP, it is possible that our dose selection based on studies in mice was not appropriate. An alternative approach I can consider is an adaptive dose-escalation study, as Hardy et al had designed when evaluating the effectiveness of IV LEV for CSE (Hardy et al. 2012). Applying this approach, if the 2 mg/kg dose does not cause adverse effects in the first cohort of animals, the following cohort would receive a higher dose that has been shown to be well-tolerated in dogs (e.g. 3 mg/mL).

Another alternative approach would be to conduct an adaptive Bayesian study design where the study can be stopped earlier if it has been determined that ALLO is much more superior or inferior compared to IV DZP. This approach may allow a smaller number of required canine patients, however it would require information on the expected efficacy of IV ALLO in the treatment of CSE. Since

the efficacy in CSE is unknown, I may use the efficacy rate of 92% from a mouse model of BZD-refractory SE while making large assumptions or conduct a smaller pilot study to better estimate the effectiveness on CSE (Zolkowska, Wu, and Rogawski 2018).

Rather than compare IV ALLO to IV DZP directly, I could compare the effectiveness of IV DZP + normal saline with IV DZP + IV ALLO. This design would be considered a placebo-controlled trial, however, given that both study drugs potentiate $GABA_A$ current, it is possible they will exhibit a synergistic effect and increase the risk for severe sedation (Ying-Qing and Rong 2001; Gunter et al. 2016). Furthermore, a placebo-controlled trial typically test for superiority rather than noninferiority, which may require a larger sample size.

If animals exhibit toxicity (i.e., strong sedation) but superior seizure protection is not obtained, I will conclude that it is unlikely that the treatment represents an improved approach to treat SE and human subjects will be spared from expensive clinical trials that may subject them to risk.

CHAPTER 3

EVALUATING THE SAFETY OF INTRAVENOUS LACOSAMIDE FOR THE TREATMENT OF ACUTE SEIZURES

3.1 Introduction

In this chapter, I will discuss the cardiac safety concerns associated with the use of intravenous lacosamide (IV LCM) in patients under critical care. The central hypothesis is that IV LCM administration increases the likelihood for PR prolongation, which is considered an event unlikely to occur due to chance alone in healthy volunteers and ambulatory patients with epilepsy (Nada et al. 2013; Mason et al. 2007). The specific aims were to investigate the relationship between PR prolongation and IV LCM administration in this vulnerable and medically-complex patient population. The objectives of this study were to 1) estimate the proportion of patients who shift from a normal to prolonged PR interval and whose PR interval increase >20% of their baseline PR interval within 24 hours of the first IV LCM administration, and 2) determine clinical covariates that may help explain the variability in PR prolongation event occurrence. First, I will provide a brief review of LCM and a rationale for the development IV LCM for the treatment of seizure emergencies, including acute seizures and SE, followed by a manuscript.

3.2 Lacosamide

3.2.1 Physicochemical Properties

Lacosamide (molecular formula $C_{13}H_{18}N_2O_3$) is a functionalized amino acid compound with a molecular weight of 250.3 g/mol and a topological polar surface area of 67.4 A^2 (National Center for Biotechnology Information 2019b). With a

logP of 0.3, its predicted water solubility is 0.465 mg/mL (The Governors of the University of Alberta 2019), and is slightly soluble in acetonitrile and ethanol.

3.2.2 Known Mechanisms of Action

Although its precise antiseizure mechanisms are unknown, *in vitro* studies have shown that LCM selectively enhances the slow inactivation of voltage-gated sodium channels (VIMPAT® [package insert] 2019; Errington et al. 2008). Lacosamide has also been shown have neuroprotective properties. *In vitro* and *in silico* studies have shown that LCM inhibits collapsin response mediator protein 2, which decreases tubulin polymerization and neurite outgrowth (Zhang and Koch 2017; Wilson et al. 2012; Wilson and Khanna 2015; Y. Wang et al. 2010). As a result, LCM has been shown to prevent axon sprouting and decrease neuron loss following trauma and status epilepticus (Wilson et al. 2012; X. Wang et al. 2018; Licko et al. 2013).

3.2.3 FDA-Approved Indications and Marketed Formulations

Lacosamide is indicated for the management of focal onset seizures in patients at least 4 years of age (VIMPAT® [package insert] 2019). The safety of the injectable formulation has not been tested in pediatrics and is only indicated for treatment of focal onset seizures in patients who are at least 17 years old. Its marketed formulations include tablets, oral solution, and injection.

3.2.4 Clinical Pharmacokinetics

Lacosamide is completely absorbed following oral administration (approximately 100% bioavailability) (VIMPAT® [package insert] 2019). An average peak plasma concentration of 8.5 μg/mL occurs approximately 1-4 hours (VIMPAT® [package insert] 2019). Its volume of distribution is 0.6 L/kg, approximating the volume of total body water, and has low plasma protein binding (<15%) (VIMPAT® [package insert] 2019). The elimination half-life is approximately 13 hours (VIMPAT® [package insert] 2019). Approximately 40% of the dose is excreted in the urine unchanged and 30% as its major O-desmethyl metabolite (VIMPAT® [package insert] 2019). CYP3A4, 2C9, and 2C19 are responsible for the formation of its O-desmethyl metabolite, which has no known pharmacological activity (VIMPAT® [package insert] 2019). VIMPAT demonstrated no clinically significant changes on the pharmacokinetics of co-administered antiseizure drugs in patients with epilepsy, including valproic acid, carbamazepine, levetiracetam, lamotrigine, topiramate, oxcarbazepine, phenytoin, phenobarbital, gabapentin, clonazepam, and zonisamide (VIMPAT® [package insert] 2019). In patients with partial-onset seizures, small reductions in lacosamide concentrations occurred when co-administered with carbamazepine, phenobarbital, or phenytoin (VIMPAT® [package insert] 2019).

3.2.5 Present State of Knowledge of Lacosamide in Status Epilepticus

Most studies and case reports on the use of LCM for SE have been with the intravenous formulation with promising results in convulsive and nonconvulsive

SE (Bauer et al. 2017; Adam Strzelczyk et al. 2017; Paquette et al. 2015; Sebastián Ortiz De La Rosa et al. 2018). Much of the evidence is from retrospective and prospective observational open-label cohort studies or case reports. One systematic review of the evidence reports an overall efficacy of 57% (out of 522 episodes) in treating SE, with comparable efficacy in convulsive and nonconvulsive SE, and decreasing efficacy with later use of LCM in the management of SE (Adam Strzelczyk et al. 2017). In the retrospective pediatrics studies, 45-100% of refractory SE cases were successfully treated when used as a third- or fourth-line agent (Arkilo, Gustafson, and Ritter 2016; Grosso et al. 2014; Shiloh-Malawsky et al. 2011; Jain and Harvey 2012; Poddar, Sharma Mbbs, and Ng 2016). There is no paucity of retrospective open-label studies in adult refractory SE. Of the few larger studies, IV LCM was successful in terminating 33-88% of refractory SE cases and was well-tolerated (C. Kellinghaus et al. 2011; C Kellinghaus et al. 2014; Sutter et al. 2013; Höfler et al. 2011; Santamarina et al. 2018; Newey et al. 2017; Garcés et al. 2014). In addition, 36-80% of prospective open-label refractory adult SE cases were successfully treated when IV LCM was used as a third- or fourth-line agent (Legros et al. 2014; Miró et al. 2013; D'orsi et al. 2016). To date, only one prospective, randomized, open-label, comparative cohort study has been published evaluating the effectiveness of IV LCM compared to IV valproate (VPA) in lorazepam-resistant SE (U. K. Misra, Dubey, and Kalita 2017). Seizure termination within one hour was no different between the two groups (63.6% LCM, 69.7% VPA), but the LCM group had a statistically insignificant lower

proportion of cases with 24-hour seizure freedom (45.5% LCM, 60.6% VPA). These results suggest that LCM is as effective as VPA in lorazepam-resistant SE and warrants the development of LCM as a treatment for established SE.

3.2.6 Rationale for Developing Intravenous Lacosamide for Treatment of SE

The unique mechanisms of action of LCM, neuroprotective properties, lack of significant drug-drug interactions, low protein binding, availability of intravenous and oral formulations, and lower risk for systemic complications make LCM an ideal agent for the treatment of seizure emergencies like SE and/or in medically-complex patients. Considering the systemic complications SE alone, employing a therapy that does not increase risk for hypotension (like phenytoin), hepatoxicity (like VPA), or respiratory depression (like barbiturates) would be highly desirable (Hawkes and Hocker 2018). Although IV LCM has shown great promise, its use is somewhat limited by its potential to cause PR prolongation and other cardiac dysrhythmias (Rudd et al. 2015; Nizam et al. 2011).

3.3 Intravenous Lacosamide Use and PR Interval Prolongation in the Critically-Ill Patient

3.3.1 Introduction

Seizures are a common occurrence in critically-ill patients. The prevalence of seizures in this population has been estimated to range between 16-27% (Koppel et al. 2001; Brandon Westover et al. 2015; Herman et al. 2015). Moreover, the presence of electrolyte abnormalities, hepatic and/or renal impairment,

requirement of mechanical ventilation, and polypharmacy (enzyme inducers/inhibitors, central nervous system depressants, and drugs that affect/maintain cardiovascular function, etc) further complicate the hemodynamic and metabolic status of critically-ill patients. Although there are a number of antiseizure drugs (ASDs) available to treat seizures in the inpatient setting, many of these drugs have a high risk for drug-drug interactions and can cause systemic complications, including hypotension, Stevens-Johnson Syndrome, and respiratory depression. Therefore, an ideal ASD for this population should be available as an intravenous (IV) formulation, have a rapid onset of action, not be highly protein bound, not induce/inhibit enzymes, and have a low risk for serious adverse effects.

Among the ASDs with available IV formulations, lacosamide (LCM) is appealing for use in critically-ill patients due to its novel mechanisms of action and low potential for drug-drug interactions. However, LCM has been associated with cardiac conduction abnormalities in the form of PR interval prolongation. In a study of 944 patients with partial-onset seizures across four oral dose groups (placebo, 200-, 400-, and 600-mg), dose-related mean increases in PR interval length were observed. Of those patients, 0.4% developed asymptomatic first-degree atrioventricular block (PR segment prolongation >0.2 msec) (Rudd et al. 2015). In addition to PR interval prolongation, LCM has been associated with serious cardiac arrhythmias including atrial fibrillation, bradyarrhythmia, and ventricular tachycardia (Shaibani et al. 2009; Krauss et al. 2010; Berei, Lillyblad, and Almquist 2018). These concerns for PR interval prolongation or other

potential cardiac issues limits the use of IV LCM in patients at high-risk for developing cardiac arrhythmias or conduction abnormalities.

Although there is information on the overall efficacy and safety of IV LCM in the critically-ill, there is a lack of detailed information on PR interval changes in this patient population (Sutter et al. 2013; Ramsay et al. 2015; Newey et al. 2017). These papers capture PR intervals before and after IV LCM administration, however details on when PR intervals were recorded are limited, or the times at which PR intervals were collected do not permit determination of association between PR interval prolongation with IV LCM exposure.

We performed a retrospective chart review to investigate the relationship between IV LCM use and PR interval prolongation in patients in an intensive-care unit (ICU) setting. Our primary objectives were to 1) estimate the proportion of critically-ill patients whose PR interval shifts from normal (≤200 msec) to prolonged (>200 msec) after receiving at least one dose of IV LCM, and 2) estimate the proportion of critically-ill patients who have a PR interval increase of >20% from baseline after receiving at least one dose of IV LCM. Our secondary objective was to evaluate clinical factors that may help explain the variability in PR prolongation event occurrence.

3.3.2 Methods

3.3.2.1 Study Design

We performed a retrospective chart review of all patients (aged 18-89 years) admitted into either a cardiac, medical, or neurological ICU following admission

at Abbott Northwestern Hospital who received at least one dose of IV LCM between October 2008 and June 2017. The study was approved by the Allina Health and University of Minnesota institutional review boards.

3.3.2.2 Inclusion Criteria

Patients must have also had at least one PR interval reading within 24 hours before and after IV LCM administration. We used the longest pre-dose and post-dose PR intervals for statistical analyses.

3.3.2.3 Determination of PR Interval Prolongation

We defined PR interval prolongation as a shift from normal (≥200 millisecond [ms]) to prolonged (>200 ms) PR interval or an increase of >20% in PR interval from pre-dose PR interval. Although an increase of >20% in PR interval is not a known surrogate marker of PR interval prolongation or cardiac arrhythmias, this cutoff was based on the observation from descriptive electrocardiographic studies of ambulatory healthy volunteers across several clinical trials that the occurrence of either event in PR interval would be considered rare and unlikely to occur by chance alone (Nada et al. 2013; Mason et al. 2007). Percent change from baseline PR interval was calculated by subtracting the pre-dose PR interval from the post-dose interval and dividing by the pre-dose interval. Proportions of PR interval prolongation and their respective 95% confidence intervals (CI) were calculated using R (Version 3.4.3).

3.3.2.4 Evaluation of Clinical Variables

A covariate analysis was performed in order to identify clinical factors that help predict PR prolongation. Variables of interest included: age upon admission, sex, race, weight, body mass index (BMI), length of stay (LOS), condition on discharge (alive/expired), serum creatinine (Scr), glomerular filtration rate (GFR), blood urea nitrogen, glucose, smoking status (never, current, former), serum electrolytes (potassium, sodium, chloride, calcium, phosphorus, magnesium, bicarbonate), anion gap, dose, total daily dose (TDD), pre-dose PR interval, post-dose PR interval, new cardiac arrhythmias, and concomitant medications (drugs known to cause PR interval prolongation or cardiac disturbances, strong and moderate CYP2C9 inhibitors, and strong and moderate CYP3A4 inhibitors). The distribution of all predictors were examined visually; those that were heavily skewed were log-normalized, while all others were normalized to the median value. Missing values for continuous predictors were imputed with the median value. Missing values for factors were left blank. Logistic regression analysis ($log\frac{\pi}{1-\pi} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots$ where π is the probability of an event occurrence, $\frac{\pi}{1-\pi}$ is the odds of an event occurrence, β_0 is an intercept, β_i is the coefficient of the predictor) was used, with PR interval prolongation coded as “1”. Forwards and backwards stepwise covariate inclusion were used. A p-value < 0.01 was considered statistically significant. Selection criteria during the model development process were based on changes in Akaike’s Information Criterion, residual unexplained variability (deviance), and parameter estimates and their standard errors.

3.3.3 Results

3.3.3.1 Study Demographics

Of the 162 patients screened, 34 were excluded due to lack of recorded pre- or post-dose PR intervals, sixteen had a pacemaker, and one was older than 90 years. Our final analysis included 111 patients. Sixty-three patients were male, 53 had epilepsy-related primary diagnoses, 87 were Caucasian, eleven were African American, fourteen expired prior to discharge, and twelve patients were on a co-medication of interest. The median pre- and post-dose PR intervals of

180 msec and 180 msec, respectively (ranges: pre-dose: 118-260 [standard devation (sd): 30] msec; post-dose: 131-320 [sd: 28] msec) (Table 3.3.3-1).

Table 3.3.3-1 Patient Demographics

Variable	Min	25th percentile	Median	75th percentile	Max	Mean	St. Dev.
Dose (mg)	50	100	200	200	400	179.27	80.19
Total Daily Dose (mg)	100	300	400	600	1200	440.09	209.20
Pre-dose PR Interval (msec)	118	160	180	200	260	182.04	30.46
Post-dose PR Interval (msec)	131	170	180	200	320	186.29	27.94
Change from Pre-dose PR Interval (msec)	-60	-10	6	20	90	4.25	26.55
Age upon admission (years)	18.18	39.46	57.23	69.84	86.37	54.20	19.28
Length of stay (days)	1.70	4.29	8.51	16.13	50.65	12.19	10.40
Serum Creatinine (mg/dL)	0.42	0.67	0.79	1.00	7.61	1.11	1.09
Estimated Glomerular Filtration Rate (mL/min)	6.95	70.78	92.74	112.64	223.35	91.68	40.45
Serum Potassium (mEq/L)	2.10	3.60	3.90	4.30	5.10	3.92	0.50
Serum Total Calcium (mg/dL)	6.60	8.20	8.70	9.00	10.20	8.62	0.65
Serum Magnesium (mg/L)	0.90	1.70	2.00	2.20	4.70	2.04	0.56
Anion Gap (mEq/L)	3	8	9	11	17	9.40	2.47
BMI	17.27	22.52	26.87	31.75	48.86	27.63	6.18
Weight (lbs)	96.00	154.40	177.50	202.15	329.87	181.36	42.87

3.3.3.2 Prevalence of PR Prolongation

Eight percent (n = 7/88, 95% confidence interval (CI): 2.3,13.6) of our patients had a shift from normal to prolonged PR interval, and 13% (n = 15/111, 95% CI: 7,20) of our patients had a >20% increase in PR interval after their first dose of IV LCM (Figure 3.3.3-1).

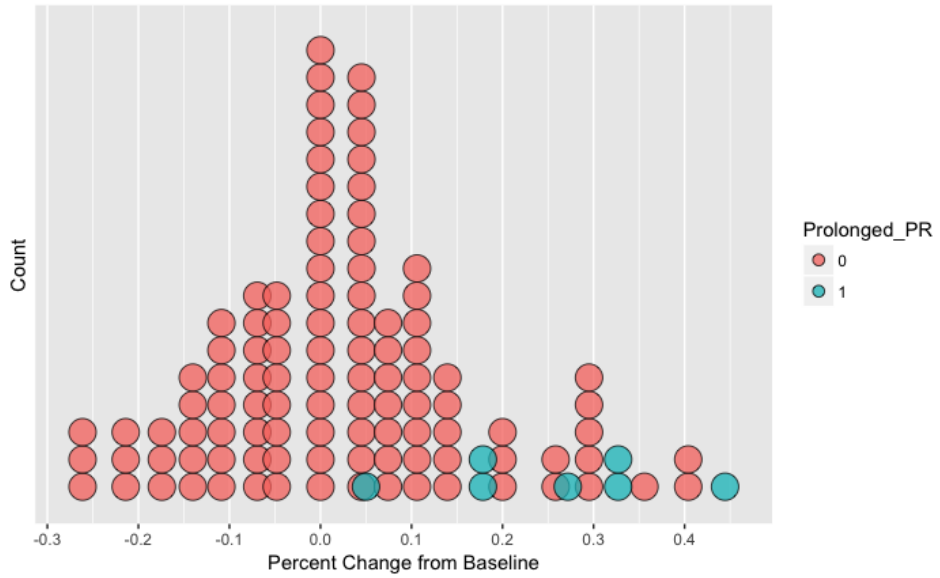

Figure 3.3.3-1 Dotplot of Primary Outcomes. Each circle represents a patient. Blue circles denote patients who experienced a PR prolongation within 24 hours following IV LCM administration.

3.3.3.3 Logistic Regression Analysis

Based on a statistical significance cut-off of <0.01, age, total daily LCM dose (TDD), and serum potassium levels were associated with PR prolongation based on trending significance (Table 3.3.3-2). After adjusting for all other covariates, as a patient increases in age by 10 years, the odds of PR prolongation increase by $e^{0.07*10}$ = 2-fold; as the TDD increases by 100 mg, the odds increase $e^{0.01*100}$ = 2.27-fold (or 127%); as the serum potassium (normalized to the median serum potassium of 3.9 mEq/L) increases by 0.25 units (equivalent to an increase in serum potassium of 0.98 to 4.88 mEq/L), the odds increase $e^{7.62*0.25}$ = 6.72-fold. Figure 3.3.3-2 is an illustration of the relationships between these covariates and

Table 3.3.3-2 Logistic Regression Results for PR Prolongation

Coefficients	Estimate	Standard Error	z-value	p-value
Intercept	-17.51	5.58	-3.14	<0.01
Age	0.07	0.03	2.28	<0.05
Total Daily Dose	0.01	0.002	2.05	<0.05
Normalized Serum Potassium	7.62	3.86	1.98	<0.05

PR interval prolongation. The cases (blue circles) tended to be either on or above the median TDD, on or above the median serum potassium, and older in age.

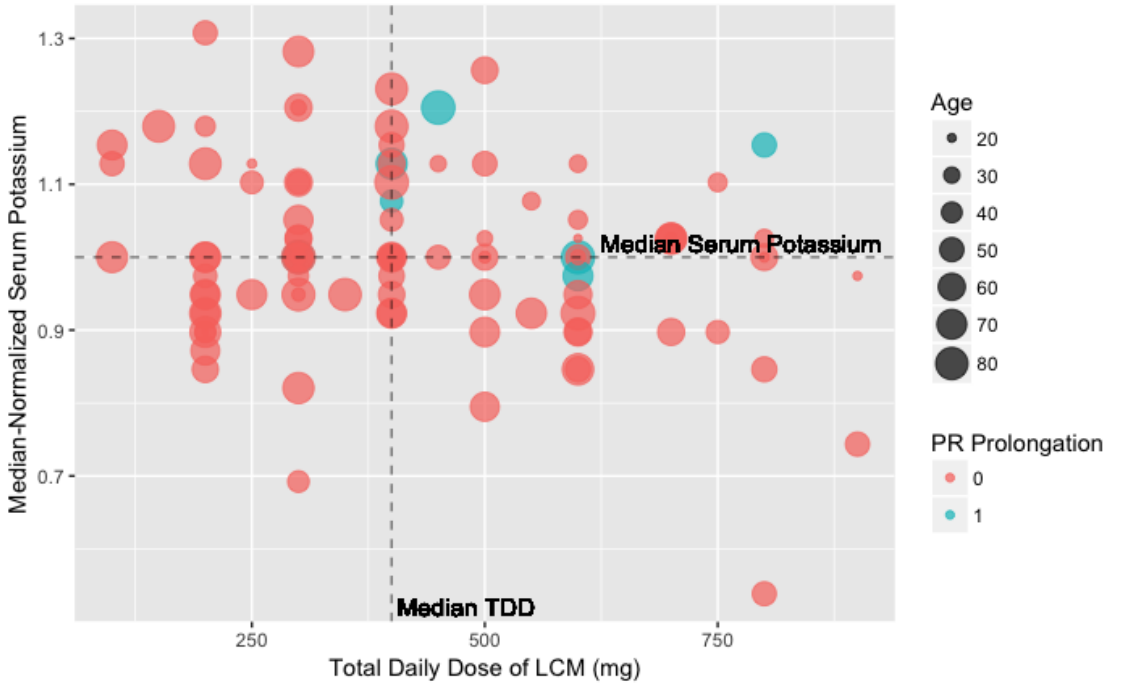

Figure 3.3.3-2 Relationships between Age, Total Daily Dose (TDD) of LCM, Median-Normalized Serum Potassium, and PR Prolongation.
The size and color of the circles denote the age (i.e. larger circle, older age) and PR prolongation (i.e. blue = shift from normal to prolonged PR interval), respectively.

On the other hand, a shorter baseline PR interval and current/former smoking status are associated with a baseline-adjusted increase in PR interval >20% following IV LCM (Table 3.3.3-3). After adjusting for all other covariates, as pre-dose PR interval increases by ten ms, the odds of having a >20% increase from baseline PR interval increases by a factor of $e^{-0.07*10} = 0.5$ (or decrease by 50%); if patient is a current or former smoker, the odds increases by

Table 3.3.3-3 Logistic Regression Results for PR Increase >20%

Coefficients	Estimate	Standard Error	z-value	P-value
Intercept	8.19	2.98	2.75	<0.01
Pre-dose PR Interval	-0.07	0.02	-3.48	<0.001
Current/Former Smoker	2.50	1.06	2.36	<0.05

a factor of $e^{2.50} = 12.2$. Figure 3.3.3-3 depicts the binomial regression of the event of a >20% increase from pre-dose PR interval on its two covariates. The regression is less steep for current/former smokers, which suggests that the odds of a >20% baseline-adjusted increase stays relatively high for current/former smokers compared to a patient who has never smoked. For example, at the same baseline PR interval of 150 msec, the odds of an event occurrence are approximately 0.4, whereas for a nonsmoker, they close to 0.

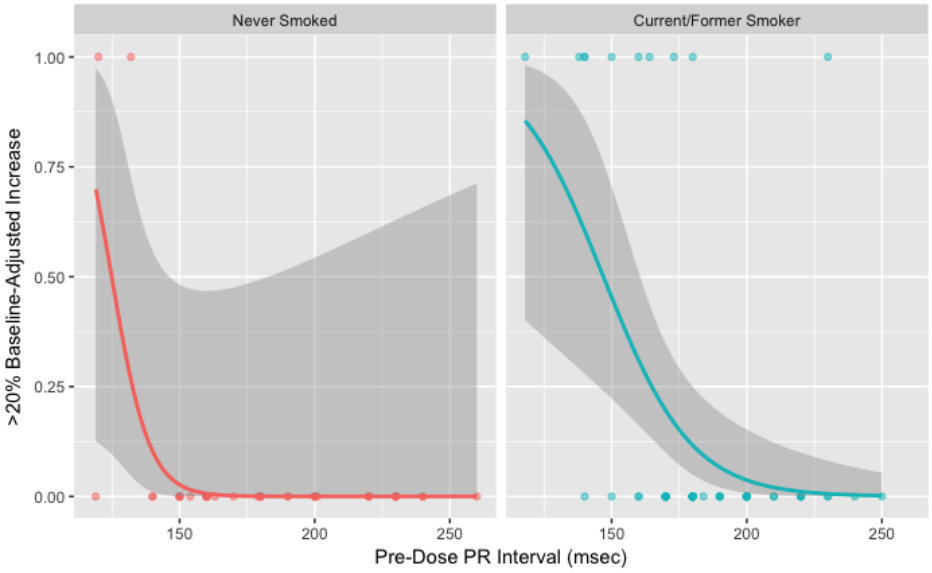

Figure 3.3.3-3 Pre-Dose PR Interval on Event Occurrence and the Effect of Smoking Status

3.3.4 Discussion

Intravenous LCM is known to be an effective and well-tolerated treatment for seizures in hospitalized adult and pediatric patients (Christoph Kellinghaus, Berning, and Besselmann 2009; Luk et al. 2012; Arkilo, Gustafson, and Ritter 2016; Ngampoopun et al. 2018; Welsh et al. 2017). While several studies have suggested that LCM is a relatively safe ASD, there are a growing number of reports describing its adverse effects on cardiac conduction. A recent (November

2018) FDA alert described new prescribing information for LCM which now contains additional serious warnings about cardiac arrhythmias, including ventricular arrythmias. Few studies have systematically evaluated LCM effect on cardiac function in the critical care setting. Our study sought to evaluate the relationship between IV LCM use and PR interval prolongation in patients in the ICU. An important finding in our study is that IV LCM use did not cause significant change in median PR interval when comparing pre- and post-drug exposure within the first 24 hours of administration. However, we observed that 8% patients had prolonged PR interval and 13% patients had PR interval increases >20% of baseline PR interval following IV LCM administration. The 8% of patients who experienced PR prolongation is greater than the previously reported prevalence of 0.4% in ambulatory patients (Rudd et al. 2015).

One of our goals was to determine if there was a specific subpopulation of patients who were more likely to experience significant increases in PR interval. Our logistic regression analyses revealed that older age, higher TDD, and higher serum potassium levels are weakly associated with PR prolongation. Many of these covariates are expected. Older age has long been associated with longer PR intervals, and there is increasing awareness of its increased risk for atrial fibrillation (Mason et al. 2007; Magnani et al. 2013; Cheng et al. 2015; Macfarlane et al. 2011). There may be confounding variables that may explain the relationship between older age and PR prolongation, including heart rate (Soliman and Rautaharju 2012). In addition, oral and IV LCM has been reported to increase PR interval in a dose-dependent manner and cause first-degree

atrioventricular block in a small number of cases (Rudd et al. 2015; Shaibani et al. 2009; Krauss et al. 2010; Ramsay et al. 2015; Nizam et al. 2011). Interestingly, higher serum potassium levels are typically associated with a lower PR interval (Noordam et al. 2019). Therefore, it would seem counterintuitive that these patients would be more likely to experience PR prolongation. However, we did not find a correlation between serum potassium levels and pre-dose PR intervals in our study sample. Therefore, at least in our sample, serum potassium levels were associated with PR prolongation independent from baseline PR interval.

With a p-value cut-off of <0.01, only pre-dose PR interval was a strong predictor of a >20% increase in PR interval following IV LCM administration. This seems rather intuitive, since a lower baseline would require a smaller increase to be considered a case. In contrast, the relationship between smoking status and PR interval increase was unexpected. The association between smoking status and a higher odds of PR interval increase >20% of baseline may be due to the effect of nicotine on cardiac conduction. Nicotine increases AV conduction and decreases the refractory period of the AV node, and is a risk factor for atrial fibrillation (Haass and Kiibler 1996; Watanabe 2018). In fact, several prospective, longitudinal cohort studies (including the Health, Aging, and Body Composition Study) also found that smoking status was a useful factor in predicting incident cardiovascular disease with increases in PR intervals (Cheng et al. 2015; Magnani et al. 2013). There may also be confounding variables that we were unable to tease out given the small sample size, such as the presence

of cardiovascular or respiratory condition, age, and sex. It would have also been of interest to assess whether these patients were had concomitant use of varenicline, bupropion, or other smoking cessation drugs.

This study is one of the largest reported series of IV LCM use in critically ill patients to date and used a systematic protocol to assess PR intervals. The limitations of our study included the retrospective design, lack of control group, and small sample size. Furthermore, we lacked information on arrhythmias in our sample. We were able to obtain new arrhythmia data in the electronic medical record in 2 out of 7 patients who had PR prolongation and 9 out of 15 patients who had a PR interval increase >20% from baseline. Of those patients, only 2 had a new cardiac arrhythmia diagnosis (atrial fibrillation, cardiac arrest, paroxysmal ventricular tachycardia, supraventricular tachycardia, unspecified atrial fibrillation, or other specified cardiac dysrhythmias). It is unclear whether these event occurrences are important predictors for cardiac dysrhythmia at this time. Finally, the absence of a control group prohibited our ability to determine whether the higher event occurrence is attributable to the drug effect, the circumstances that lead to ICU admission, and/or the complex interaction of co-morbidities in this patient population.

3.3.5 Conclusions

The prevalence of PR interval prolongation and of PR interval increase of >20% of baseline following IV LCM administration in the critically-ill patient population are 8% and 13%, respectively. These event occurrences are higher than what

has been reported in the literature for ambulatory patients taking LCM oral tablets and more common than expected in healthy volunteer Phase I clinical trials. Larger, controlled, prospective studies are needed to confirm the prevalence of these events and impact of clinical variables on PR interval changes.

3.4 Design of a Prospective Observational Study of PR Prolongation in Critically-Ill Patients on Intravenous Antiseizure Therapy: Focus on Intravenous Lacosamide

Despite the available evidence-based recommendations for management of *established* SE, 12-50% of cases are resistant to current therapies (Malamiri et al. 2012; Agarwal et al. 2007; W. B. Chen et al. 2011; U. Misra, Kalita, and Maurya 2012; Lyttle et al. 2019; Dalziel et al. 2019; Gujjar et al. 2017; Mundlamuri et al. 2015; Nene et al. 2019). My long-term goal is to find a safe drug for the treatment of established SE. Fortunately, since the its FDA approval, IV LCM has shown promise in the acute treatment of seizures and refractory SE. Its neuroprotective properties, lack of significant drug-drug interactions and lower risk for systemic complications make it a particularly attractive agent for the treatment of seizure emergencies and use in critically-ill patients.

The development path for potential approval of IV LCM for the treatment of seizure emergencies includes conducting clinical trials demonstrating its safety and effectiveness. However, its use in established SE is limited by the lack of strong evidence (i.e. prospective, blinded, randomized comparative or noninferiority trials) and the concerns for its safety in patients with cardiac

dysfunction. Therefore, there remains a need to further evaluate the safety of IV LCM, with particular focus on cardiac rhythmicity. The following proposed study has a primary objective of comparing the prevalence of PR prolongation following IV LCM administration in critically-ill patients to matched controls. The results of this study will help address concerns for its use in the critically ill population and/or seizure emergencies.

3.4.1.1 Study Rationale

The retrospective study revealed that patients in critical-care who have received at least one dose of IV LCM are more likely to experience PR interval prolongation compared to healthy individuals and ambulatory patients with epilepsy who have taken oral LCM. The major limitations from my retrospective study is the lack of a control group and missing data in the medical charts. Without a control group, I was unable determine whether the increased prevalence of PR interval prolongation was due to IV LCM use alone or to the circumstances of the critically-ill population (e.g. co-morbidities or reason for ICU admission). Without data on new arrhythmia diagnoses, I was unable to determine the clinical significance of PR prolongation following IV LCM administration. Thus, a prospective observational study can be conducted to address these issues.

3.4.1.2 Study Objective

The primary objective of this study is to compare the proportion of critically-ill patients who develop PR prolongation within 24 hours immediately following IV LCM administration to the proportion of critically-ill patients matched by admission date, ICU, age, and sex who were administered an IV antiseizure drug (excluding LCM).

3.4.1.3 Study Population

All patients (aged 18-89 years) admitted into either a cardiac, medical, or neurological ICU following admission at a tertiary care hospital will be screened over a ten year period. Consent and enrollment will occur if it is likely that a patient will require an IV antiseizure drug (i.e. fosphenytoin, phenytoin, valproic acid, levetiracetam, brivaracetam, LCM, or lamotrigine) or if the patient received one 24 hours prior to enrollment (e.g. if drug needed to be administered quickly in an emergent situation). Patients must have at least one PR interval reading within 24 hours before and after initial antiseizure drug (and/or IV LCM) administration.

3.4.1.4 Study Design

A prospective observational study will be performed. Control patients will be matched to IV LCM patients by admission date, ICU, age (+/- 5 years), and sex to a ratio of 3:1 (control patients to IV LCM patients). Control patients are defined as patients who required at least one IV antiseizure drug and did not receive IV

LCM during the studied hospital admission. Oftentimes, a patient may have had at least one IV antiseizure drug before and/or after requiring the use of IV LCM. For those patients, antiseizure drugs used within 24 hours prior to and after IV LCM administration will be recorded for covariate testing. Control patients will be matched on the number of antiseizure drugs prior to IV LCM administration. For example, if a patient received IV LCM after trying two other IV antiseizure drugs, three control patients of the same sex and age, from the same admission date, in the same type of ICU, who have also received three antiseizure drugs will be matched. The PR intervals prior to and after the use of the third antiseizure drug will be recorded and used for statistical analyses. After the study period, a data extraction team will be utilized to pull data of interest from electronic medical records.

3.4.1.5 Sample Collection

All of the following will be extracted from the electronic medical records: age upon admission, sex, race, weight, body mass index (BMI), length of stay (LOS), condition on discharge (alive/expired), serum creatinine (Scr), glomerular filtration rate (GFR), blood urea nitrogen, glucose, smoking status (never, current, former), serum electrolytes (potassium, sodium, chloride, calcium, phosphorus, magnesium, bicarbonate), anion gap, dose, total daily dose (TDD), pre-dose PR interval, post-dose PR interval, new cardiac arrhythmias, concomitant medications (drugs known to cause PR interval prolongation or cardiac disturbances, strong and moderate CYP2C9 inhibitors, strong and

moderate CYP3A4 inhibitors, and smoking cessation drugs), and antiseizure drugs used within 24 hours prior to and following the first dose of IV LCM (or antiseizure drug in the equivalent sequence of IV LCM in matched patient).

3.4.1.6 Data Analysis Plan

The longest PR interval before and after IV antiseizure drug and/or IV LCM administration will be used for statistical analyses. PR interval prolongation will be defined as a shift from a normal (≤200 millisecond [ms]) to prolonged (>200 ms) PR interval. Proportions of PR interval prolongation and their respective 95% confidence intervals (CI) will be calculated using R (Version 3.4.3). Continuous demographic and baseline variables such as sex, age, weight, laboratory values (i.e. basic metabolic panel), and number of previous antiseizure drugs within 24 hours prior to IV LCM treatment will be tested between treatment groups using a two-sample *t*-test or Mann-Whitney test; categorical variables such as sex, race, primary diagnosis, and smoking status will be tested using a Chi-square test. If any of the subgroups are expected to be less than 5 in count, Fisher's exact test will be used.

The distribution of all predictors will be examined visually; those that are heavily skewed will be log-normalized, while all others will be normalized to the median value. Missing values for continuous predictors will be imputed with the median value. Missing values for factors will be left blank. Logistic regression analysis ($log\frac{\pi}{1-\pi} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots$ where π is the probability of an event occurrence, $\frac{\pi}{1-\pi}$ is the odds of an event occurrence, β_0 is an intercept, β_1 is the

coefficient of the predictor) will be used, with PR interval prolongation coded as "1". Stepwise covariate method will be used to determine significant covariates with a forward-inclusion criterion of $p<0.05$ and a backwards-inclusion criterion of $p<0.01$. Selection criteria during the model development process will be based on changes in Akaike's Information Criterion, residual unexplained variability (deviance), and parameter estimates and their standard errors.

3.4.1.7 Challenges and Limitations

One of the biggest challenges noted from the initial retrospective study is the small number events (PR interval prolongation). This affected my ability to search for significant predictors. To offset this, I can lengthen the time over which patient data will be collection period and recruit more tertiary hospital centers within the same health system (to minimize system-wide differences in practice). Another challenge is missing data in the electronic medical record. It was found to be relatively common for screened patients to be missing either a pre- or post-dose PR interval, laboratory values, and/or new diagnoses of arrhythmias. It was also difficult to determine whether these data are missing data completely at random, missing at random, and missing not at random. For example, are patients with missing arrhythmia diagnosis data arrhythmia-free (missing not at random), or was the arrhythmia not charted correctly (missing at random or missing completely at random), or were neither of possibilities listed above not true but data are somehow missing (missing completely at random). To minimize this limitation, ICU staff can be trained to collect all data of interest for all patients

who receive IV antiseizure drugs, especially PR intervals and arrhythmia diagnosis data.

3.4.1.8 Expected Results and Alternative Approaches

I expect that the proportion of critically-ill patients who experience PR prolongation following IV LCM administration will not be statistically different from those who do not receive IV LCM. It is possible that the co-morbidities and complications that lead a patient to intensive care may contribute cardiovascular and autonomic stress, resulting in a longer PR interval. This is reflected in the observed high average PR interval at baseline in my retrospective study and that which was reported by Luk et al (Luk et al. 2012). Given these assumptions, I expect that the proportions of PR prolongation in critically-ill patients will be statistically different (and greater) than those from healthy volunteers or ambulatory patients with epilepsy. An alternative approach to answering the same question would be to conduct larger retrospective study and include matched controls as mentioned above. However, the challenge with retrospective studies is that one would have less control over what data will be available in the medical records. Finally, given the medically-complex circumstances, ethical considerations limit the study of IV LCM in critically-ill patients who have conditions for which IV LCM is not indicated, in those who do not need an IV antiseizure drug, and in those whose hospitalists would not use IV LCM as first choice therapies in.

CHAPTER 4

DEVELOPMENT OF AN INVESTIGATIONAL INTRAVENOUS FORMULATION OF TOPIRAMATE FOR THE TREATMENT OF REFRACTORY STATUS EPILEPTICUS

4.1 Introduction

Topiramate is a drug used for focal onset or generalized tonic-clonic seizures and for adjunctive treatment for seizures associated with Lennox-Gastaut Syndrome for patients at least 2 years of age (Chapter 1.2.1.3). An intravenous formulation in the development phase and could be beneficial as an adjunctive treatment for SE based on its multiple mechanisms of action and low potential for drug-drug interactions as described in more detail below. The objective of my work presented here was to characterize the pharmacokinetics and pharmacodynamics of IV TPM in dogs. The specific aims were to 1) characterize the pharmacokinetics of IV and oral TPM, 2) describe changes in iEEG activity, and 3) simulate dosing regimens that would have the highest likelihood of achieving plasma concentrations associated with SE response to inform the design of a clinical trial in canine SE. In this chapter, I will provide a brief review of topiramate and a rationale for the development IV TPM for the treatment of SE, followed by published manuscript which describes my research.

4.2 Topiramate

4.2.1 Physicochemical Properties

Topiramate (molecular formula $C_{12}H_{21}NO_8S$) is a sulfamate-substituted fructose analog with a molecular weight of 339.36 g/mol and a topological polar surface area of 124 A^2 (National Center for Biotechnology Information 2019c). With a logP of -0.8, its water solubility is 9.8 mg/mL, and is soluble in most alkaline solutions (pH 9-10) containing sodium hydroxide/phosphate and freely soluble in acetone, dimethylsulfoxide, and ethanol. Its saturated solution has a pH of 6.3.

Taken together, these properties suggest TPM is highly soluble in neither water nor lipids, and transport across biological membranes is likely not by passive diffusion.

4.2.2 Known Mechanisms of Action

Although its precise antiseizure and migraine prophylaxis mechanisms are unknown, preclinical studies suggest that at pharmacologically relevant concentrations, topiramate inhibits voltage-gated sodium channels, enhances activity at $GABA_A$ receptors subtypes, blocks AMPA/kainate glutamate receptors, and antagonizes carbonic anhydrase isozymes II and IV (TOPAMAX [package insert] 2019). Furthermore, there is evidence of neuroprotective properties in animal models of limbic SE, methylphenidate-induced toxicity, hypoxia-ischemia, and stroke (Niebauer and Gruenthal 1999; Motaghinejad et al. 2017; Schubert et al. 2005; Liu et al. 2004; Cha et al. 2002). More specifically, these studies illustrated that TPM reduced neuronal cell death in the area of the brain most susceptible to seizures and improved cognitive function. The multiple mechanisms of action and neuroprotective properties of TPM make it an attractive agent for treating SE.

4.2.3 FDA-Approved Indications and Marketed Formulations

Topiramate is indicated for monotherapy and adjunctive therapy in patients at least 2 years of age with partial onset or primary generalized tonic-clonic seizures, for patients at least 2 years of age with seizures associated with

Lennox-Gastaut syndrome, and for migraine prophylaxis in patients 12 years of age or older (TOPAMAX [package insert] 2019). Its marketed formulations are limited to tablets, immediate-release sprinkle capsules, and extended-release capsules. Extemporaneous compounding of an oral suspension using the immediate-release tablets can be made using a mixture of Ora-Sweet and Ora-Plus (Allen 2017).

4.2.4 Clinical Pharmacokinetics

Topiramate is well-absorbed after oral administration (TOPAMAX [package insert] 2019). An average peak plasma concentration of 27 μg/mL occurs approximately 2 hours following 400 mg multiple oral dose administration every 12 hours (FDA CDER 2004). Clinically relevant plasma concentration range of up to 33 μg/mL (FDA CDER 2004). TPM is 15-41% plasma protein bound, and its mean elimination half-life is approximately 21 hours (TOPAMAX [package insert] 2019). TPM exhibits dose-proportional PK over 100-400 mg dose range (FDA CDER 2004). In 19 studies of human PK and PD submitted by the sponsor, TPM was found not to be extensively metabolized (FDA CDER 2004). The major route of elimination is by renal excretion, with 80% excreted unchanged in 24 hours (FDA CDER 2004). However, when changed from PHT or CBZ + TPM therapy to TPM monotherapy, TPM oral clearance was reduced by 50% (Bourgeois 2007; R. Sachdeo et al. 2002; R. C. Sachdeo et al. 1996). As a result, two-fold lower plasma TPM concentrations have been observed (Contin et al. 2013; Yamamoto et al. 2017). Although the enzymes responsible for its metabolism have not been

fully characterized, it is possible that the increase in oral clearance may be due to increased expression of intestinal efflux transporters like P-glycoprotein (Wang-Tilz et al. 2006; Atasayar et al. 2016).

In dogs, 90% of TPM is excreted unchanged over 96 hours (Caldwell 2005). However, TPM is metabolized more extensively in dogs compared to people. The most dominant metabolic pathway was found to be hydrolysis at the 2,3-O-p-isopropylidene group.

4.2.5 Present State of Knowledge of Topiramate in Status Epilepticus

Cases reported in the literature have suggested that TPM was well-tolerated and effective in stopping more than 50% of cases with different types of SE (Shorvon and Ferlisi 2012; Wasterlain and Chen 2008). The first case reports of enteral TPM used in adult refractory and super-refractory SE were published in 2002 and 2003 (Reuber, Evans, and Bamford 2002; Towne et al. 2003; Bensalem and Fakhoury 2003). After these reports, much of the literature on TPM use in refractory and super-refractory SE were from use in pediatrics, which showed termination of SE in 77% (17/22) of cases (Kahriman et al. 2003; Perry, Holt, and Sladky 2006; Blumkin et al. 2005; Akyıldız and Kumandaş 2011). Since then, a few retrospective chart analyses have been published and one prospective open-label non-randomized clinical trial (Stojanova and Rossetti 2012; W. Kim et al. 2011; Synowiec et al. 2011; Hottinger et al. 2012; Asadi-Pooya et al. 2015). In total, the retrospective studies reported success in terminating refractory SE in

70% cases (61/87). However, there are a number of confounders, including the number of other ASDs administered, the place in SE management where TPM was used (e.g. administration as the third ASD versus sixth ASD), or the effect of the combination of medications rather than TPM alone (Hottinger et al. 2012; Synowiec et al. 2011). In addition, in many of these studies, TPM tablets were crushed to a powder and mixed with water (Towne et al. 2003; Synowiec et al. 2011). Given that TPM is not highly soluble in water, it is possible that the lack of clinical response may be due to limited bioavailability of this "formulation." In the prospective study, TPM was administered as an adjunctive therapy via nasogastric tube 30 mins after administration of second-line therapy (phenytoin) (Asadi-Pooya et al. 2015). Five of the twenty (25%) patients successfully responded (SE termination within 24 hours following TPM introduction without modification of concomitant ASDs). Although prospective, this study lacks a control, and it is difficult to distinguish whether seizures stopped due to the addition of phenytoin, topiramate, or both. Although there is a lack of randomized, double-blinded, controlled trials evaluating TPM safety and effectiveness for either refractory or super-refractory SE, the enteral TPM appears to show promise in the treatment of SE.

4.2.6 Rationale for Developing Intravenous Topiramate for Treatment of SE

The multiple mechanisms of action of TPM, especially the potentiation of GABA-ergic inhibition via a benzodiazepine-insensitive pathway and antagonization of glutaminergic AMPA/kainate receptors (White et al. 2000), would be particularly

useful when considering SE pathophysiology. That is, TPM has the potential to be effective after the internalization of benzodiazepine-sensitive $GABA_A$ receptors and increased externalization of AMPA/NMDA receptors in the synaptic cleft.

Considering all of its potential benefits, the use of TPM for acute seizure emergencies like SE is limited by its route of administration. Despite an oral absolute bioavailability of approximately 100%, peak plasma concentrations are achieved at approximately two hours following a solid oral dose (Clark, Kriel, Leppik, White, et al. 2013a; Clark, Kriel, Leppik, Marino, et al. 2013; Cipla USA 2017). Although the time to peak concentration following the typical TPM preparation comprised of crushed TPM tablets in water is unknown, the earliest time to SE termination following enteral TPM was twelve hours (Towne et al. 2003; Stojanova and Rossetti 2012), with the highest cumulative response rate at 72 hours (Hottinger et al. 2012; Stojanova and Rossetti 2012; Synowiec et al. 2011; W. Kim et al. 2011; Bensalem and Fakhoury 2003). Further, these results are likely confounded by the various factors, including inconsistent doses and titration schedules, lack of controls, and possible disrupted gastrointestinal motility due to SE or other acute co-morbidities (Deane et al. 2019). Therefore, an intravenous formulation would enable a more rapid loading of TPM that would bypass the need for enteral movement and the variability in gastrointestinal absorption. This would allow for attainment of higher peak concentrations in a shorter length of time compared to the same dose given enterally in this patient population. Furthermore, an intravenous route of administration would remove

the need for an enteral feeding tube (e.g. nasogastric or gastrostomy tubes), and enable TPM use earlier on in the management of SE and prior to the need for mechanical ventilation (i.e. third-line therapy).

4.3 Intravenous Topiramate: Pharmacokinetics and Effect on Electroencephalograph Activity in Dogs with Naturally-Occurring Epilepsy[1]

4.3.1 Introduction

Status epilepticus (SE) is defined as a condition characterized by abnormally prolonged seizures that can lead to long-term consequences, including permanent neuronal injury (Trinka et al. 2015). SE has been reported to have an incidence between 2.5-59% in dogs with idiopathic epilepsy, and 32% in dogs with secondary epilepsy (Saito et al. 2001; Platt and Haag 2002; Monteiro et al. 2012). In dogs that have had at least one episode of SE, overall mortality rates (primarily from euthanasia) were 32%-38% (Saito et al. 2001; Zimmermann et al. 2009). In humans, SE occurs with an incidence between 0.04-0.06% in the United States, and its complications result in an overall mortality rate of 22% (DeLorenzo et al. 1995). While benzodiazepines are the standard first line of care for SE in both dogs and humans (Michael Podell 1995; Brophy et al. 2012), approximately one third fail to respond to first line therapy (Hocker et al. 2013). There remains a need for safe alternatives for early and rapid first- and/or second-line therapy of SE to reduce the probability of recurring seizures,

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minimize associated complications, and improve patient outcomes.

One of the barriers to developing new treatments for SE is the experimental model used to find and evaluate investigational therapies. Oftentimes in rodent models, epilepsy is induced by chemical or electrical insult and may not be truly representative of epilepsy pathophysiology (Pitkänen and Mcintosh 2006). Dogs with naturally-occurring epilepsy have been proposed as appropriate models to examine new antiepileptic therapies prior to human trials (Coles et al. 2015). Canine epilepsy is strikingly similar to the human condition in both disease presentation and response to treatment. Holliday et al. demonstrated that intracranial electroencephalograms (EEGs) of dogs and humans during focal onset seizure are indistinguishable (Holliday, Cunningham, and Gutnick 1970). Moreover, studies of antiseizure drugs (ASDs), such as fosphenytoin and levetiracetam, have shown comparable efficacy in both dogs and humans for SE (Coles et al. 2015; Hardy et al. 2012).Given these similarities, assessing new therapies for SE in dogs will facilitate drug development and increase the chance of successful translation for both canine and human SE.

Among the newer ASDs with injectable formulations, topiramate (TPM) is an attractive candidate for evaluation in the treatment of SE. TPM is a second-generation, broad-spectrum ASD that inhibits of voltage-gated sodium channels and enhances gamma-aminobutyrate (GABA) activity at specific $GABA_A$ receptor subtypes (Topiramate [package insert] Cipla 2017). TPM also has mechanisms of action that differ from those exhibited by current therapies, including

antagonizing AMPA/kainate glutamate receptors, and inhibiting specific carbonic anhydrase isozymes.

Our group has studied the pharmacokinetics (PK) of a novel intravenous (IV) TPM formulation in humans. However, the pharmacokinetics of IV TPM and its effect on EEG has not been characterized in dogs. This study evaluated TPM in dogs with naturally occurring epilepsy to characterize its PK and effect on EEG activity. The aims of this study were to 1) characterize TPM PK following an IV and oral dose of TPM, 2) evaluate effect of TPM on intracranial EEG (iEEG) features, and 3) simulate doses to attain target concentrations of 20-30 µg/mL.

4.3.2 Methods

4.3.2.1 Study Animals and Safety Monitoring

Five dogs with naturally-occurring epilepsy were used in this study. Three of the dogs have uncontrolled seizures despite being on antiseizure maintenance regimens. Approval was obtained through the Institutional Animal Care and Use Committee of the University of Minnesota prior to the initiation of the study. The dogs were housed at the University of Minnesota's Veterinary College. Each dog was previously implanted with a device which wirelessly transmits iEEG recordings (Davis et al. 2011; Coles et al. 2013). Dogs were monitored continuously via iEEG and video for five to ten days to obtain baseline data, and throughout the study for vomiting, diarrhea, and lethargy prior to and for 90 minutes after drug administration, and at each blood sampling time. In the event of a seizure emergency (seizure lasting >5 minutes) or repetitive seizures (2+

seizures within 1 hour, or 3+ seizures within 4 hours), the on-call veterinarian received an automated text message and confirmed the seizure activity using remote video monitoring. The rescue therapy protocol consisted of midazolam 12 mg administered as a single IM dose.

4.3.2.2 Study Drug

For this study, a stable-isotope labeled TPM compound containing six ^{13}C, resulting in a mass 6 units greater than the unlabeled molecule was used for the IV formulation (10 mg/mL in 10% Captisol®). This formulation was manufactured by the University of Iowa under Good Manufacturing Practices and has been licensed to Ligand/CuRx Pharmaceuticals. Unlabeled TPM tablets (25 mg) purchased from the University of Minnesota Veterinary Pharmacy (Cipla USA Inc) were used for the oral treatment arm. Using a labeled IV formulation and non-labeled oral tablets allowed us to simultaneously administer both formulations and characterize TPM pharmacokinetics by each route. This approach also reduces inter-occasion variability caused by dosing on different days and/or times (Marino et al. 2012).

4.3.2.3 Dose Rationale

Based on reports of doses associated with efficacy in human SE, we estimated corresponding target plasma TPM concentrations of 20-30 µg/mL. A previous single IV dose study in one dog reported TPM concentrations from which we calculated an apparent volume of distribution (Vd) of 0.6 L/kg (Streeter et al.

1995). Using this Vd, we estimated that IV doses of 10 and 20 mg/kg would produce initial concentrations (C_0) of approximately 16 and 32 µg/mL, respectively.

4.3.2.4 Study Design

Low dose IV/oral TPM study: Four dogs were used in this study (ID 1-4, Table 1). Two of the four dogs were on ASD maintenance regimen including phenobarbital (PB). Each dog was fasted overnight prior to receiving a 10 mg/kg dose of stable-labeled IV TPM infused over 5 minutes. One hour following the IV bolus, each dog also received a 5 mg/kg dose of unlabeled oral TPM. This delay in oral administration was by design to allow evaluation of the IV dose on iEEG for one hour after dosing. Blood samples were collected from an indwelling catheter prior to dosing and at 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 9 hours following the IV bolus.

High dose IV TPM study: Three dogs were used in this study (ID 3-5, Table 1). One dog was on PB maintenance therapy. Each dog was fasted overnight prior to receiving a 20 mg/kg dose of stable-labeled IV TPM infused over 5 minutes. Blood samples were collected from an indwelling catheter prior to dosing and at 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 9 hours following the IV bolus.

Diazepam (DZP) positive control: IV diazepam (0.5 mg/kg) was administered to two dogs who were having uncontrolled seizures (ID 1 & 2) during an interictal period as a positive control as it has been shown to elicit iEEG change.

4.3.2.5 Plasma TPM Measurements

A 250 µL sample of whole blood was aliquoted for TPM analysis. The remaining blood was placed on ice and plasma was separated. All samples were immediately frozen (-20° C) until analysis. Each dog was fed no sooner than 2 hours after the oral dose. A high-performance liquid chromatography-mass spectrometry (HPLC-MS) method developed and validated at the Center for Orphan Drug Research was used to measure TPM concentrations in dog plasma. Seven calibration standards (run in triplicate) and nine quality control standards (low, medium, high run in triplicates) were prepared in plasma. Study, calibration, and quality control samples were extracted using methyl tert-butyl ether. TPM and stable-labeled TPM were analyzed using the Hewlett Packard Agilent 1100 Model G1946 liquid chromatography mass spectrometry detection system and Agilent ChemStation software. The analytes were separated using a Zorbax C18 column (150 mm x 3.0 mm, 3 µm) and the mobile phase consisted of an ammonium acetate buffer and methanol. The quantization was performed using the selective ion monitoring in the negative mode, with deuterated TPM (d10) as the internal standard. The mass-to-charge ratios were 338 m/z and 244 m/z for TPM and stable-labeled TPM, respectively. The calibration curves were linear ($r^2 = 0.998$) in the concentration range of 0.05–50 µg/mL for TPM and

0.05-10 µg/mL for stable-labeled TPM in plasma. The limit of detection and quantitation were 0.05 ng/mL and 0.05 µg/mL, respectively. The precision for both TPM and stable-labeled TPM ranged from 3-6%, and accuracy values were between 95-114% and 86-105%, respectively.

4.3.2.6 Pharmacokinetic Analysis

Topiramate concentration-time data were analyzed using non-compartmental analysis (Phoenix WinNonLin, version 6.4, Pharsight Corporation, Mountain View, CA, USA). Pharmacokinetic parameter values included maximum concentration (C_{max}), time at which maximum concentration is achieved (t_{max}), elimination rate half-life ($t_{1/2}$), and the area under the time-concentration curve (AUC_{INF}) calculated using the equation $AUC = \int_{t=0}^{t=\infty} Cp * dt$ (where Cp is the plasma TPM concentration) and a linear-log trapezoidal method. Oral bioavailability (F%) was calculated using the equation $\mathrm{F}\ (\%) = \frac{\mathrm{AUC(oral)} * \mathrm{Dose(IV)}}{\mathrm{AUC(IV)} * \mathrm{Dose(oral)}} \mathrm{x}\ 100$. Clearance (CL) and Vd were calculated using the equations $CL = \frac{Dose * F}{AUC}$ and $CL = k_e * Vd$, respectively, where k_e is the elimination rate constant. Concentration-time profiles were created using GraphPad Prism 7 (Version 7.0a, GraphPad Software, Inc., La Jolla, CA, USA).

PK parameters were also determined using population compartmental modeling (Phoenix Non-Linear Mixed Effects software, version 1.3, Pharsight Corporation, Mountain View, CA, USA). First order conditional estimation extended least squares method was used throughout the model building process. One- and two-compartment models were evaluated. A proportional error model

for between subject variability was used. Both additive and multiplicative error models for residual variability were evaluated. The best fit model was determined using visual inspection, goodness of fit plots, weighted residual plots, weighted sum of squared residuals, Akaike's Information Criterion, and precision of model parameters.

The presence of a CYP3A4-inducing co-medication (such as PB) was evaluated as a covariate for its influence on TPM clearance. The relationship of the covariate and TPM clearance was modeled by the equation $Cl = tvCl * e^{dCl} * e^{\eta Cl}$, where Cl is the clearance from the central compartment, tvCl is the typical value of the clearance from the population, dCl is the estimated value of the inducer effect, and ηCl is the between-subject variability of clearance. A covariate was considered statistically significant if inclusion of the covariate resulted in a decrease in the objective function value (OFV) of at least 6.64 ($p < 0.01$, χ^2, degree of freedom = 1). The final model was used to simulate of 5-, 10-, and 15-minute infusions IV TPM at doses ranging from 10-30 mg/kg.

4.3.2.7 Electroencephalographic Analysis

Sixteen electrode channels were continuously sampled at 399.6 Hz. A band-pass filter was applied to create 6 frequency bands: delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-25 Hz), low gamma (25-40 Hz), and high gamma (40-120 Hz). In order to evaluate differences in EEG features, energy of each electrode within each frequency band was calculated in 1-second intervals by summing the square of the EEG signal amplitude within the 1 second window.

The average energy level was calculated for three 15-minute ranges: starting from 15-minutes pre-dose to dosing, from dosing to 15-minutes post-dose, and from 15-minutes post-dose to 30-minutes post-dose. The difference between averaged energy levels at pre-dose and each post-dose interval were calculated. *P*-values were generated by the Kruskal-Wallis test comparing the averaged energy level from pre-dose to the two averaged energy levels post-dose.

4.3.3 Results

4.3.3.1 Demographics and Adverse Events

Demographics of the dogs are represented in Table 4.2.3-1. No adverse events were observed for either dose group throughout the course of the study.

Table 4.3.3-1 Animal Demographics

Subject	Weight (kg)	Breed	Seizure Type	Co-medications
1	33	Coonhound Mix	Focal and grand mal seizures	Levetiracetam, zonisamide, phenobarbital
2	29	Labrador Retriever Mix	Cluster seizures	Levetiracetam, zonisamide, phenobarbital, potassium bromide
3	15	Beagle	None	N/A
4	29	Coonhound Mix	None	N/A
5	35	Coonhound Mix	Focal and grand mal seizures	Phenobarbital

4.3.3.2 Non-Compartmental Analysis

The concentration-time profiles of plasma ^{13}C-TPM following the low- and high-dose IV infusions are shown in Figure 4.3.3-1. Pharmacokinetic parameter estimates using non-compartmental analysis are summarized in Table 4.3.3-2. TPM clearance was greater and elimination half-life shorter in dogs receiving chronic PB. The clearance was 0.5-0.7 L/hr/kg versus 0.1 L/hr/kg and elimination half-life 0.5-1 hour versus 3.7-5 hours in dogs with and without PB, respectively, suggesting hepatic enzyme induction by PB. Clearance, volume of distribution, and elimination half-life were similar for both dose groups studied. AUC_{INF} approximately doubled as dose doubled suggesting dose-proportional pharmacokinetics.

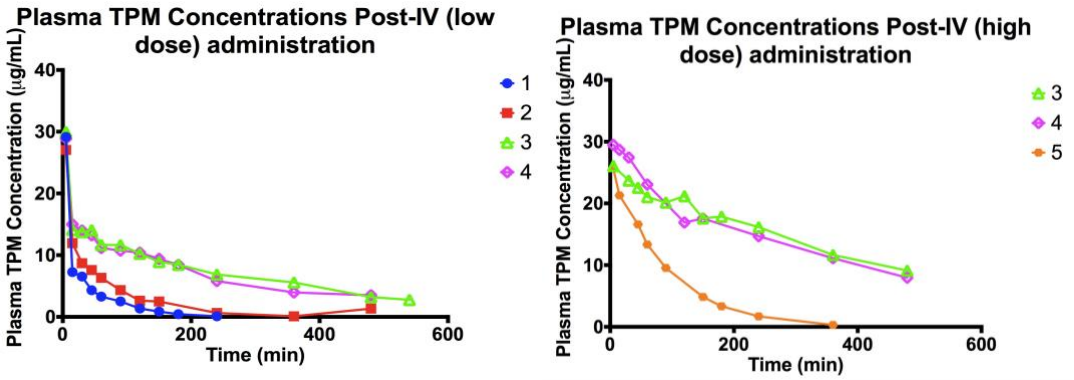

Figure 4.3.3-1 Plasma-Concentration Time Profiles Following IV Administration of a Low (10 mg/kg) and High (20 mg/kg) dose of stable-labeled TPM

Table 4.3.3-2 Pharmacokinetic parameter estimates generated from non-compartmental analysis following IV administration

ID	Group	$t_{1/2}$ (hr)	C_1 (µg/mL)	AUC_{INF}_obs (µg*hr/mL)	V (L/kg)	CL (L/hr/kg)
1	LOW	0.47	29.2	13.7	0.50	0.73
2	LOW	0.75	27.1	20.6	0.53	0.49
3	LOW	3.71	30	83.5	0.64	0.12
4	LOW	4.05	28.9	101	0.58	0.1
3	HIGH	4.99	26.1	194	0.74	0.1
4	HIGH	4.52	29.5	176	0.74	0.11
5	HIGH	0.95	25.7	38.4	0.71	0.52

LOW = 10 mg/kg dose. HIGH = 20 mg/kg dose. $t_{1/2}$: elimination half-life; C_1: first measured concentration; AUC_{INF}_obs: observed area under the curve from time 0 to infinity; V: volume of distribution; CL: clearance.

Plasma TPM concentration-time profiles following oral administration are depicted in Figure 4.3.3-2. C_{max} following oral administration ranged between 1.9-2 µg/mL at 1-1.5 hours (T_{max}), with a $t_{1/2}$ between 1.7-2 hours in the two dogs on PHB. In the two dogs not on PB, a C_{max} of 4.7-5.5 µg/mL at 0.5-1 hour was observed, with a $t_{1/2}$ of 4 hours. Individual oral bioavailability ranged between 61-102%. These results are summarized in Table 4.3.3-2. Similar to the IV

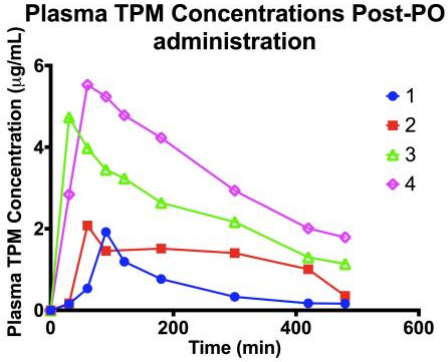

Figure 4.3.3-2 Plasma-concentration time profiles following administration of a single 5 mg/kg TPM oral dose

Table 4.3.3-3 Pharmacokinetic parameter estimates generated from non-compartmental analysis following PO administration

ID	$t_{1/2}$ (hr)	T_{max} (min)	C_{max} (µg/mL)	AUC_{INF}_obs (µg*hr/mL)	V (L/kg)	CL (L/hr/kg)	F (%)
1	2.02	90	1.92	4.73	2.13	0.73	69.2
2	1.66	60	2.08	10.5	1.16	0.49	102
3	3.98	30	4.73	25.7	0.69	0.12	61.7
4	4.08	60	5.53	36.9	0.58	0.1	73.0

Volume and clearance were adjusted for bioavailability. $t_{1/2}$: elimination half-life; T_{max}: time at peak concentration; C_{max}: peak concentration, AUC_{INF}_obs: observed area under the curve from time 0 to infinity; V: volume of distribution; CL: clearance; F: bioavailability.

administration, the two dogs on PHB exhibited higher clearance rates, and consequently, shorter half-lives compared to the two dogs not on PB.

4.3.3.3 Population Compartmental Analysis

A two-compartment model with first-order elimination best fit the TPM concentration data following IV administration (Figure 4.3.3-2). Parameter estimates are provided in Table 4.3.3-4. A systematic bias in clearance based on

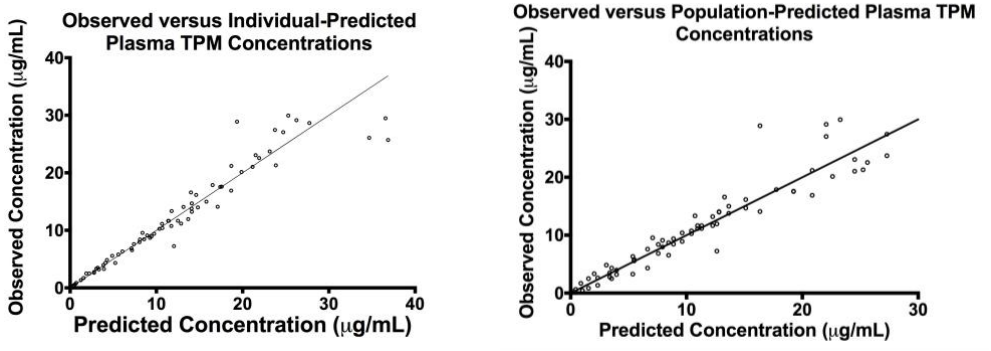

Figure 4.3.3-3 Observed versus predicted individual and population concentrations

dose was observed. The inclusion of whether the dog was on an enzyme-inducing co-medication as a covariate resulted in a decrease in the OFV from the base model (difference in OFV = 25) and an improvement in the goodness of fit

Table 4.3.3-4 Pharmacokinetic parameter estimates from a population compartmental analysis following an intravenous TPM (low and high doses)

Model Parameter		**Estimate**	**Stderr**	**CV%**	
Fixed Effect	tvV (mL/kg)	376	72.4	19.2	---
	tvV2 (mL/kg)	298	56.0	18.7	---
	tvCL (mL/(kg*min))	1.84	0.08	4.52	---
	tvCL2 (mL/(kg*min))	21.0	9.37	44.7	---
	dCL	1.73	0.13	7.66	---
Random Effect		**Estimate**	**Stderr**	**RSE%**	**Shrink%**
	BSV_V	0.08	0.02	24.6	9.3
	BSV_{Cl}	0.02	0.01	53.4	9.18
	Residual error, CV%	14.9	1.71	11.5	---

tvV: Typical value of volume of distribution from central compartment; tvV2: Typical value of volume of distribution from peripheral compartment; tvCL: Typical value of clearance from central compartment; tvCL2: Typical value of intercompartment clearance; dCL: Effect of PB presence on CL; BSV: between-subject variability; CV%: coefficient of variation; RSE%: relative standard error

plots and precision of parameter estimates. Therefore, the effect of an enzyme inducer on clearance was included in the final model. The presence of PB is estimated to affect TPM clearance by a factor of 5.64. Except for peripheral compartment clearance, all model-fitted parameters were estimated with good precision with all coefficients of variation below 25%. A multiplicative error model best described the residual error with an estimate of 15%, which is consistent with analytical error. Visual predictive check plots (Figure 4.3.3-4) illustrated the observed data percentiles fall within the 90% (5%-95%) model-predicted intervals.

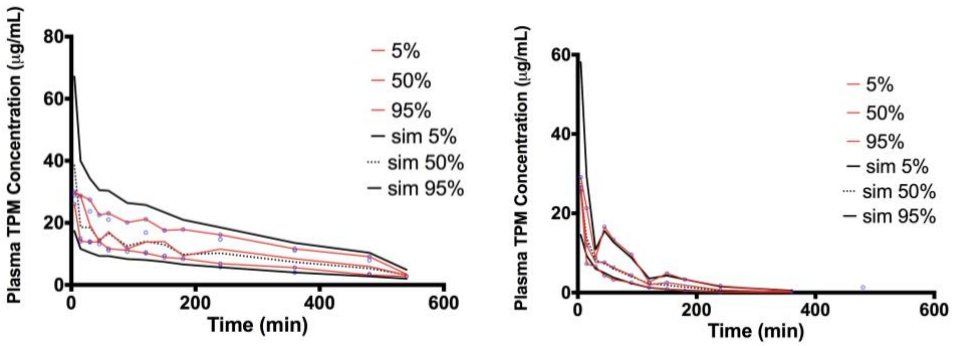

Figure 4.3.3-4 Visual predictive check plots of a population model for dogs not on PB and dogs on PB, respectively. Red and black lines represent the observed and simulated quantiles, respectively.

4.3.3.4 Simulation Analysis

Using the final model above, various infusion rates and doses were simulated (Figure 4.3.3-5). For dogs not on enzyme-inducing co-medications, simulated time-concentration profiles suggest that a 5-minute infusion of 20 mg/kg would achieve target concentration range of 20-30 µg/mL at 30-minutes post-dose. However, in dogs on enzyme-inducing co-medications, a dose between 25-30 mg/kg infused over 5 minutes would be required to attain the same target range

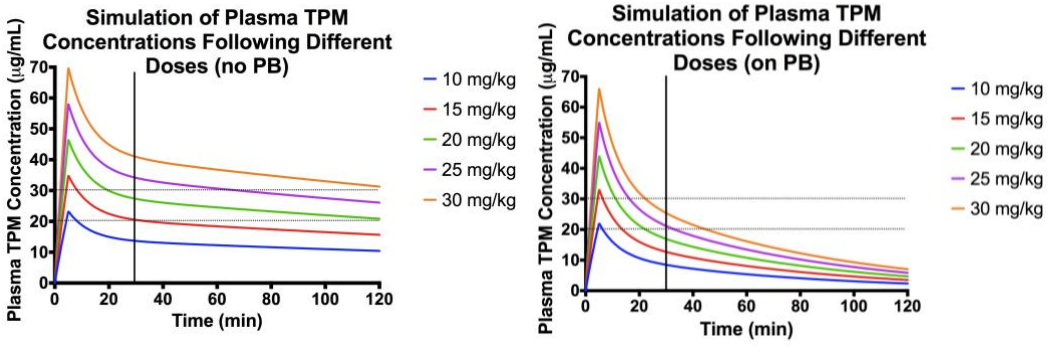

Figure 4.3.3-5 Simulated Plasma Concentration Time Profiles Across Varying Doses Infused Over 5 Minutes in a dog not on PB and a dog on PB. Here, the yellow band is the desired range, and the black vertical line denotes 30 minutes post-dose.

4.3.3.5 Electroencephalographic Analysis

EEG data of good quality were not attained for all animals due to electrical malfunctioning of the electrodes and/or data cards as some of the devices had been implanted for up to 5 years. In the dogs with a functioning device for EEG acquisition, intravenous TPM produced EEG changes shortly after the infusion which continued in the subsequent 40-60 minutes (Figure 4.3.3-6). Statistically

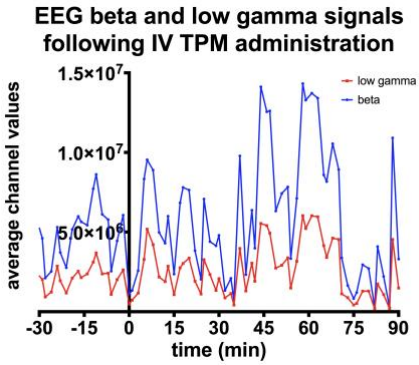

Figure 4.3.3-6 EEG normalized signals from beta and low gamma frequency bands averaged over 1-minute intervals in one dog

significant positive energy differences in all 6 frequency bands across all 16 channels were seen comparing the pre-dose time (-15 to 0 mins) to both post-

Table 4.3.3-5 Difference between energy levels averaged across low gamma and beta frequency bands before and after IV TPM in one dog

Time Ranges (min)		**Frequency Bands**					
Pre-dose	**Post-dose**	**delta (1-4 Hz)**	**theta (4-8 Hz)**	**alpha (8-12 Hz)**	**beta (12-25 Hz)**	**low gamma (25-40 Hz)**	**high gamma (40-120 Hz)**
(-15, 0)	(0, 15)	16631.60*	3997.93*	1250.46*	1214.93*	342.61*	265.21*
(-15, 0)	(15, 30)	17765.57*	3710.96*	1127.09*	972.23*	333.26*	220.43*

dose times (0 to 15 mins and 15 to 30 mins) (Table 4.3.3-5). Intravenous DZP also increased energy in frequencies >4 Hz (most prominently in beta and gamma frequency bands), and significantly decreased delta frequency energy in most channels (data not shown).

4.3.4 Discussion

This study is unique in that it evaluated both the pharmacokinetic and pharmacodynamics aspects of TPM in dogs with naturally occurring epilepsy. This is also the first study characterizing the effect of PB on TPM pharmacokinetics in dogs. We observed a 5.6 times greater CL in dogs on PB compared to those who were not. In addition, the three dogs on PB exhibited lower peak TPM plasma concentrations following oral dosing. This increase in clearance suggests that there is induction of hepatic enzymes that metabolize TPM. This has also been noted in humans, although the effect of enzyme induction in humans is more modest (~145% increase in clearance) (Clark, Kriel, Leppik, White, et al. 2013b). To explain this discrepancy, Caldwell et al found that while 82% of TPM is excreted unchanged in the urine in humans, only 28% is excreted unchanged in dogs (Caldwell 2005).Phenobarbital is a known inducer of CYP 3A4, the major enzyme responsible for TPM metabolism. A recent study evaluating the effects of chronic administration of PB on the pharmacokinetics of levetiracetam in dogs with epilepsy found similar results (Muñana, Nettifee-Osborne, and Papich 2015). Potential drug-drug interactions should be taken into consideration when dosing TPM in both dogs and humans. Dose adjustments

are likely needed when TPM is used in conjunction with chronic enzyme-inducing or enzyme-inhibiting co-medications in dogs.

Although a limitation of the study is the small number of animals used to construct the population PK model, the utility of the model is to simulate concentration profiles for dogs both on and not on enzyme-inducing co-medications. It has been noted that dogs respond to many of the same ASD therapies as humans. With the use of simulations, we can predict drug exposure from different dosing strategies and determine the optimal dosing regimen to attain the same concentrations that are considered therapeutic in human SE, as we did successfully in dogs with a PK study and a randomized clinical trial with IV fosphenytoin (Coles et al. 2015; Patterson et al. 2015). Based on case reports of TPM oral suspensions used to treat refractory SE, our goal target concentration range was 20-30 µg/mL. Our simulations suggest that these doses should be used in designing future a clinical trial in canine SE.

The significant changes between pre-dose EEG energy levels and those up to 30 minutes after IV TPM administration suggest sufficient and timely diffusion into the brain, suggesting that IV TPM may be a good candidate for treatment of seizure emergencies. Benzodiazepines in both rodent and dog model show significant increases in energy in frequencies greater than 4 Hz and decreases in delta frequency energy (Table 4.3.3-5), which is expected from prior studies. After IV TPM administration, we also see changes in these frequencies at 15 minutes. These observations suggest IV TPM may be beneficial for the treatment of status epilepticus.

In conclusion, IV TPM doses of 10 and 20 mg/kg infused over 5 minutes were shown to be safe and tolerable in dogs. Concurrent administration of PB increased the clearance of TPM approximately 5.6-fold. Simulations suggest that doses of 20 and 25 mg/kg of IV TPM are necessary to achieve a target concentration between 20-30 µg/mL in dogs not on PB and dogs on PB, respectively. A key strength of this study is the use of animals with naturally occurring epilepsy. The results of this study provide information on optimizing TPM therapy for future studies of canine SE, which will subsequently guide the design of IV TPM clinical trials of human SE. Future work includes conducting a phase II/III efficacy study in canine SE using the dose strategy determined from the PK modeling results of this study.

Disclosures

Drs. Cloyd and Leppik are paid consultants for CuRx Pharmaceuticals. Dr. Cloyd also receives payments from a licensing agreement between the University of Minnesota and Ligand Pharmaceuticals. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Acknowledgements

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research. We also acknowledge and thank Andrea Eckert for her care of the animals and sample collection.

4.4 Development of Intravenous Topiramate for the Treatment of Refractory Status Epilepticus

There is currently insufficient evidence to guide refractory SE therapy due to the rarity of the condition and difficulty in the interpretation of findings due to the complex interaction of drugs used in parallel and co-morbidities at this later stage of SE. Early results of a multinational, prospective audit of 488 patients with refractory and super-refractory SE reported that 74% of cases recovered from RSE, 22% died, and 4% had treatment withdrawn due to futility (Ferlisi et al. 2015). Although anesthetic agents are useful in suppressing seizures, they are associated with a higher risk of systemic complications death independent of underlying medical conditions (Sutter et al. 2014). There is an unmet need for better control of refractory SE, ideally before the need for burst-suppression. The multiple mechanisms of action and neuroprotective properties of TPM make it an ideal drug to use when resistance to the recommended antiseizure drugs has occurred.

Because oral TPM is an FDA-approved drug, the development of IV TPM has two possible routes: 1) temporary replacement for oral TPM therapy by showing that the IV formulation can be safe and bioequivalent to an oral product, or 2) conduct controlled safety and efficacy trials for a new indication, such as treatment for acute seizures and/or seizure emergencies. Single-doses of 100

mg IV TPM infused over 15 minutes has already been shown to be bioequivalent to 100 mg oral TPM and safe in healthy volunteers and a PK and safety study in patients with epilepsy or migraines taking oral TPM (Clark, Kriel, Leppik, Marino, et al. 2013; Clark, Kriel, Leppik, White, et al. 2013b). The next step for this direction could be to conduct a single- and multiple-ascending dose study to assess the safety and tolerability of higher doses of TPM and longer exposure of IV TPM. This information is needed for its development as a temporary replacement for oral TPM therapy considering patients with epilepsy can be prescribed at least the maximum recommended daily dose of 400 mg divided into two doses. For example, in case reports where TPM preparations were used to treat super-refractory SE, patients were administered up to 1000 mg TPM per day (Brigo, Bragazzi, and Igwe 2017). In addition, it would be useful to know the maximum tolerated duration of IV therapy for this same indication. This study would not replace the need for a safety and effectiveness clinical trial in SE, but these data would inform the safety of IV TPM for the treatment of seizure emergencies.

The following proposed studies have a primary objective of characterizing the safety of higher IV TPM doses and its effectiveness in treating refractory SE in dogs.

4.4.1 IV Topiramate for the Substitution of Oral Topiramate

4.4.1.1 Study Rationale

Multiple dose studies have not been conducted to evaluate the safety of larger single doses of IV TPM and multiple infusions of IV TPM with respect to infusion site adverse effects.

4.4.1.2 Study Objective

The primary objective of this study is to characterize the safety and pharmacokinetics of single- and multiple-ascending doses (SAD/MAD) of IV TPM. Pharmacokinetic parameters estimated from the SAD studies will be used to simulate exposures in the MAD studies, and adjust infusion rates if necessary. The results of these studies will inform labelling recommendations on the maximum duration of replacement therapy and the maximum tolerated dose that could be safely infused in situations of emergent seizures.

4.4.1.3 Study Population

Equal proportions of male and non-pregnant and non-breastfeeding female patients >18 years of age stable on TPM at daily doses between 300-400 mg will be recruited for the single ascending (SAD, six patients per dose cohort and two planned cohorts). Patients who are stable on TPM at daily doses between 200-400 mg will be recruited for the multiple ascending dose studies (MAD, six patients per dose cohort and three planned cohorts). Patients must not be on any interacting co-medications (a comprehensive “excluded concomitant medication”

will be provided). Being stable on oral TPM is defined as having received the same dosing regimen for at least the past 4 weeks, and without plans to adjust the regimen during the study period. The SAD/MAD study can be conducted in parallel.

4.4.1.4 Study Design

The SAD study will be an open-label study. Patients will receive their half of their daily TPM dose (150-200 mg) as a *stable-labeled* infusion over 15 minutes (stable-labeled IV TPM described in Clark et al 2013). All patients will be instructed to take the remaining half of their daily dose as oral tablets 12 hours after study drug infusion, and as prescribed every day after for the remainder of the 96-hour study period. Infusion sites will be monitored for adverse effects such as tenderness and swelling. If two of six patients have a DLT, the next cohort will receive the same dose. If only one patient experiences a DLT, the next cohort will receive the next higher dose of 200 mg IV TPM infused over 15 minutes. The study will repeat until three or more people per SAD level have a DLT or 200 mg IV TPM has been completed. If the 150 mg single dose is determined to be safe, simulations will be done to predict exposures for the Cohort 4. This will also be done following the 200 mg single dose.

The MAD (Cohorts 3-5) study will also be an open-label study. The first MAD cohort (Cohort 3) will receive 100 mg of *stable-labeled* IV TPM over 15 minutes twice daily for two weeks. In Cohorts 4 and 5, patients will receive half of their daily dose of IV TPM infused over 15 minutes twice daily for two weeks if

the corresponding infusion was determined to be safe during the SAD study. The study will repeat until three or more people per MAD level have a DLT or a daily dose of 400 mg IV TPM has been completed.

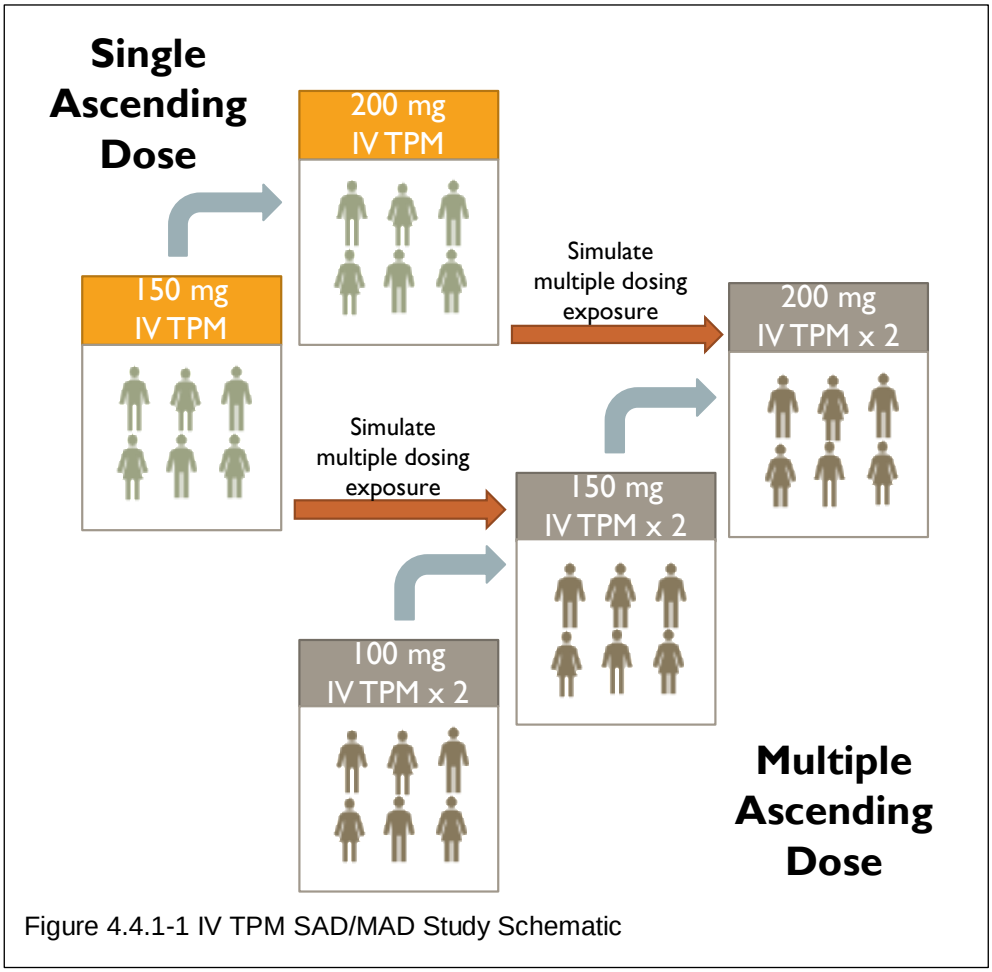

Figure 4.4.1-1 IV TPM SAD/MAD Study Schematic

4.4.1.5 Sample Collection

For the SAD studies, blood samples pre-injection, and at 15- and 30-minutes, 1, 2, 4, 6, 12, 24, 48, 72, 96 hours following IV infusion will be collected to determine the pharmacokinetics. For the MAD studies, blood samples pre-injection, and at 1,12, 24, 48, 72, 96, 120, 240, and 336 hours following IV infusion will be collected to determine the steady-state pharmacokinetics.

4.4.1.6 Pharmacokinetic Analysis

Plasma TPM concentration-time data will be analyzed using non-compartmental analysis and population compartmental modeling approaches. First order conditional estimation extended least squares method will be used throughout the model building process. The best fit model will be determined using visual inspection, goodness of fit plots, weighted residual plots, weighted sum of squared residuals, Akaike's Information Criterion, and precision of model parameters. PK parameters estimated from the SAD cohorts will be used to predict exposures in the MAD cohorts and to adjust infusion rates, if needed. Infusion rates may need to be adjusted at higher dose levels to ensure the maximum concentration (Cmax) does not exceed those observed following oral TPM dosing. For example, if the Cmax following 200 mg dose is higher than predicted based on data from previous studies (Clark, Kriel, Leppik, Marino, et al. 2013; Clark, Kriel, Leppik, White, et al. 2013a), the infusion length can be increased.

4.4.1.7 Challenges and Limitations

A challenge to this particular study may be the number of dropouts due to the neuropsychiatric adverse effects such as decreased working memory, verbal fluency, attention and psychomotor speed, especially in the MAD cohorts (Ngee Lim et al. 2016; Ahmed et al. 2014).

4.4.1.8 Expected Results and Alternative Approaches

Based on the demonstrated bioequivalence between the 100 mg oral and IV formulations, I expect to see a bioequivalence up to the single 200 mg doses. Furthermore, based on the safety and tolerability seen in dogs at up to 20 mg/kg infused over 5 minutes (using the FDA HED, this would be equivalent to a 775 mg dose in an average 70 kg adult), I expect to see drowsiness, mild sedation, and impaired psychomotor functions at 400 mg daily dose, which may or may not be more severe than what the patient has already experienced on stable therapy of oral TPM. This may also not be of great concern, considering patients who will be requiring replacement of oral therapy will likely be in an acute care setting, where these impairments can be monitored. Single- and multiple-ascending dose studies are common first-in-human studies used to determine the maximum tolerated dose and to perform preliminary food/formulation effect and drug-drug interaction testing (Shen et al. 2019). This study is designed to show the safety/tolerability of higher doses and of multiple infusions. The results of the MAD study will be used to determine whether there is an upper limit to the duration of IV replacement therapy. An alternative approach is to conduct the SAD/MAD study in healthy volunteers. This would eliminate some confounders such as co-medications, co-morbidities, and/or bias in recruiting patients who require higher doses of TPM (who may have more severe forms of epilepsy). However, with healthy volunteers, one can ethically compare the adverse effects of IV TPM infusion with a placebo infusion.

4.4.2 Design of a Clinical Study of Intravenous Topiramate for the Treatment Established Status Epilepticus in Dogs

4.4.2.1 Study Rationale

I previously demonstrated that 20 mg/kg IV TPM infused over 5 minutes was safe, well-tolerated, and able to attain therapeutic concentrations for at least 20 minutes following the start of infusion in dogs with naturally-occurring epilepsy. Although I observed changes iEEG power within 15- and 30-minutes of IV TPM infusion, I still lack the evidence to conclude that IV TPM is able to terminate CSE. Therefore, a double-blinded, randomized, controlled study is needed to evaluate the safety and effectiveness of IV TPM for the treatment of established CSE. The results of this study would be used to inform the design of a clinical trial in human established SE.

4.4.2.2 Study Objective

The primary objective for this study is to demonstrate the effectiveness and safety of IV TPM as a treatment for established CSE is as effective and as safe as IV LEV.

4.4.2.3 Study Population

Dogs (5-40 kg in body weight) admitted into an emergency/urgent veterinary medical clinic with a clinical diagnosis of convulsive status epilepticus defined as continuous convulsions lasting >5 min, or 2 or more recurrent convulsions without regaining consciousness between seizures within the last 12 hours and

seizures are continuing to occur or likely to reoccur without recovery in between, have received adequate doses of a BZD either before arriving in the hospital or after a dose in the hospital, and *have a recurrent seizure prior to consenting*. Canine patients may be excluded from the study for the following reasons: owner not wishing to participate, anoxic cause for status epilepticus, or metabolic cause for status epilepticus (i.e. must have normal blood glucose, calcium, bilirubin, etc. on admission bloodwork).

4.4.2.4 Study Design

A double-blinded, randomized, multicenter non-inferiority study in canine patients with SE who have failed adequate rounds of benzodiazepines (Figure 5.4.2-1). The study involves the collection of one blood sample immediately post-infusion,

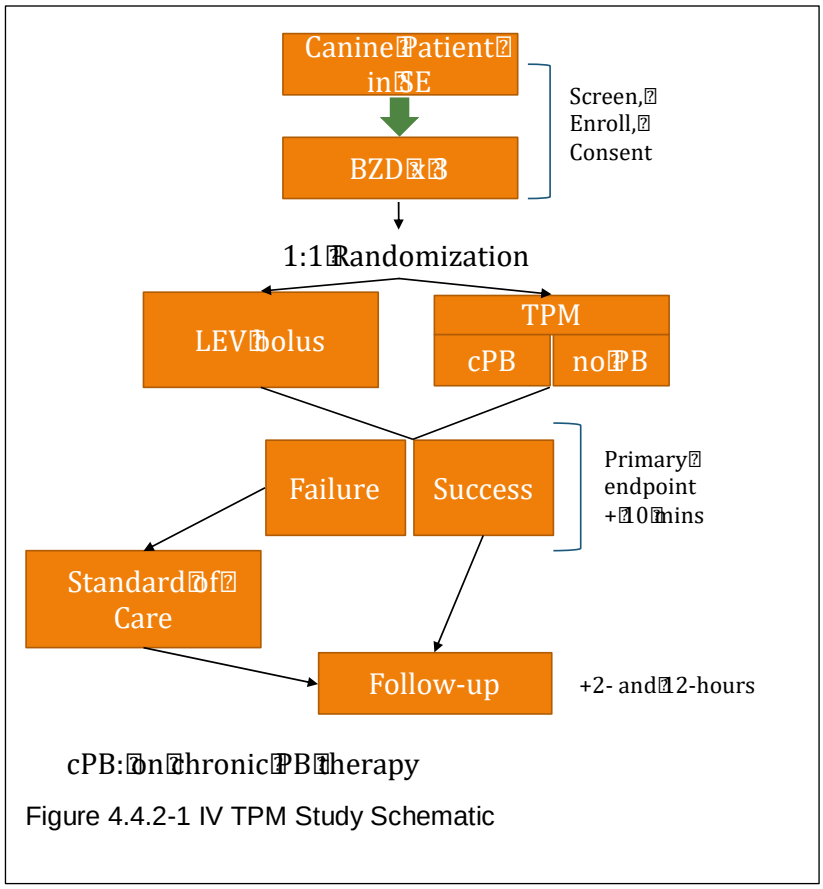

Figure 4.4.2-1 IV TPM Study Schematic

one 10 minutes after the start of study drug infusion, and one at 2 hours after the start of study drug infusion (at measurement of primary endpoint).

4.4.2.5 Endpoints

4.4.2.5.1 Primary Endpoint

Termination of seizure within 2 hours of infusion without recurrence of seizures within 12 hours of dosing. Clinical cessation of SE consists of absence of clinical seizures and improving responsiveness. Absence of apparent seizures will be determined clinically. Responsiveness will be determined by patient's response to verbal command or noxious stimuli. This study design was modeled after a prospective, double-blinded, randomized, placebo-controlled study assessing the effectiveness of IV FOS terminating CSE within 2 hours without seizure recurrence in 12 hours (Patterson et al. 2015).

4.4.2.5.2 Secondary Endpoints

Safety/tolerability (degree and duration of ataxia and sedation, heart rate, blood pressure, EKG monitoring, SpO_2 measurement, need for intubation) within 2 hours of study drug infusion, seizure cessation at 2 hours after drug administration, need for rescue therapy, time to next seizure, 12-hour responder rate, discharge status, presence of subtle CSE via EEG monitoring (when available), and plasma drug concentrations at pre-infusion, 10 minutes, and 2 hours after the start of study drug infusion. The collection of these secondary endpoints will not only inform on the safety of the study infusions, but also how drug exposures may affect the primary and secondary endpoints.

4.4.2.5.3 Blinding/Unblinding and Randomization

Patients, veterinary emergency department study team members, PIs, and clinical coordinating centers are blinded to the treatment assignment. Emergency unblinding may be required if the treating team feels that a patient's care requires knowledge of what study drug was given. Emergency unblinding will not be performed within 2 hours of the start of study drug infusion with the exception of veterinarian judgment that it is necessary for the safety or care of the patient or because of unanticipated situations accommodated by study procedures. The PK study PIs and laboratory scientists will know the assay results but will remain blinded to response until the completion of the study. Drug concentration data will not be disseminated until the completion of study.

Randomization for this study will be 1:1 between 20 mg/kg IV TPM and 60 mg/kg IV LEV.

4.4.2.6 Sample Size

The primary objective of this study is to demonstrate that the number of patients whose seizures terminate within 2 hours of study drug infusion without seizure recurrence in 12 hours in the IV TPM group is not inferior to that in the IV LEV group by more than a noninferiority margin of 10%. The null hypothesis of inferiority will be tested using a one-sided test with an 80% power and a significance of 5% (one-sided probability of a type I error of 0.05). IV LEV demonstrated a 24-hour responder rate of 56% in a prospective, double-blind, randomized placebo-controlled study of IV LEV and IV saline for the treatment of

CSE (Hardy et al. 2012). In this study, once a seizure occurred in the emergency department, canine patients were given an IV BZD followed immediately by either IV LEV or normal saline, and success was defined as seizure termination without recurrence within 24 hours. The sample size required is 340 per treatment arm (a total of 680 canine patients), which takes into account a 10% inflation to account for loss of follow up, protocol deviation (inclusion/exclusion criteria violation), and/or repeat enrollment of the same subject.

4.4.2.7 Study Drug

IV TPM (10 mg/mL in 10% Captisol®, manufactured by Ligand Pharmaceuticals) and IV LEV (commercially available as a 100 mg/mL injectable solution, diluted with normal saline to formulate a 30 mg/mL solution) will be used for this study.

4.4.2.7.1 Dose Rationale

A 20 mg/kg TPM infused over 5 minutes in dogs will attain plasma concentrations associated with successful termination of refractory SE in people. Although my simulations suggest that a 20 mg/kg IV TPM dose would only stay above the target concentrations for 20 minutes after start of drug infusion in canine patients chronically taking PB, in order to preserve a double-blinded study, only one dose of IV TPM can be used. The chronic use of PB or other enzyme-inducing co-medications will be recorded and used as a stratification in the statistical analysis. The corresponding dose for LEV is 60 mg/kg infused over 5 minutes, representing the standard of care dose for treatment of BZD-refractory SE. The

concentrations were chosen so that volumes of the two study drugs would be identical based on weight. A Dosing Chart will be provided to enable veterinary clinicians to administer the same volume of either drug based on kg bodyweight.

4.4.2.8 Study Protocol

Dogs arriving at the study centers for emergency treatment of seizures will be considered for enrollment. If they meet the inclusion criteria, and informed consent is obtained, dogs will be entered into the study and assigned a randomized, blinded treatment. A central-line catheter will have been placed at this time per standard of care protocol. After consent is obtained, the canine study patient will receive the randomized treatment: either 20 mg/kg IV TPM infused or 60 mg/kg IV LEV infused over 5 minutes. An aggressive rescue treatment plan, based on the standardized treatment protocol in place at each institution, will be initiated if the canine patient's convulsions do not diminish 10 min after the completion of the infusion or completely stop by 15 min or if motor seizure activity re-occurs within 12 hours. All subjects entered into the randomization phase will be observed for at least 12 hours after treatment with the study drug and the owners will receive $1500 towards their veterinary care. Appropriate tests will be performed to diagnose the cause of the CSE. If a dog does not survive, a post-mortem exam will be requested.

4.4.2.9 Sample Collection

Blood samples will be collected from the central-line catheter pre-infusion, and at 10 minutes and 2 hours following study drug infusion.

4.4.2.10 Data Analysis Plan

All canine patients who receive study medication will be included in the analysis and stratified based on the use of chronic enzyme-inducing medications such as PB. Continuous demographic and baseline variables such as sex, age, weight, laboratory values (complete blood count, serum chemistry, and bile acids), number of previous BZDs prior to study treatment, and duration of SE and/or number of seizures prior to arrival, will be tested between treatment groups using a two-sample *t*-test or Mann-Whitney test; categorical variables such as sex, neuter status, breed (if known), history of epilepsy diagnosis, seizure etiology, and seizure type (if history of epilepsy) will be tested using a Chi-square test. If any of the subgroups are expected to be less than 5 in count, Fisher's exact test will be used.

The primary endpoint will be compared between TPM and LEV groups using the Chi-square test.

Secondary endpoints including the time to next seizure will be compared between groups using a log rank test. Seizure cessation at 2 hours after drug administration, need for rescue therapy, 12-hour responder rate, discharge status, and presence of subtle CSE via EEG monitoring will be compared between groups using Chi-square test. In addition, these binary secondary endpoints will be compared across clinic sites using the Cochran-Mantel-Haenszel test.

4.4.2.10.1 Pharmacokinetic Analysis

TPM and LEV concentration-time data will be analyzed using non-compartmental analysis and population compartmental modeling approaches. First order conditional estimation extended least squares method will be used throughout the model building process. The best fit model will be determined using visual inspection, goodness of fit plots, weighted residual plots, weighted sum of squared residuals, Akaike's Information Criterion, and precision of model parameters. Covariates of interest will include presence of co-medications (such as bromide, imepitoin, etc), sex, neuter status, breed, age, weight, serum laboratory values (particularly liver function tests), and clinic sites.

4.4.2.11 Challenges and Limitations

The challenges and limitations of this study are highly similar to those mentioned previously in section 5.2.2.10. The additional challenge of this particular study is recruitment. Due to the more advanced stage of SE, it would not be unexpected for owners to feel that additional treatment may be futile. To offset this, a stipend will be rewarded to be put towards the cost of hospitalization.

4.4.2.12 Expected Results and Alternative Approaches

I anticipate that IV TPM will prove to achieve seizure cessation within 2 hours of dosing without seizure recurrence within 12 hours as effectively as IV LEV, and that it will be as safe as IV LEV. This will lead to consideration of IV TPM as a treatment for CSE and will encourage human clinical trials. If IV TPM does not exhibit a noninferior effect compared with IV LEV, it is possible that our dose

selection was not appropriate, and perhaps a higher dose may have been necessary. An alternative approach to account for this would be to introduce an adaptive dose-escalation design, adding a higher dose if the initial dose appears safe but inadequate. This approach was used in a study evaluating the effectiveness of IV levetiracetam for the treatment of CSE and acute repetitive seizures (Hardy et al. 2012). If animals exhibit toxicity and/or seizure protection is not obtained, I will conclude that it is unlikely that the treatment represents an improved approach to treat established SE and human subjects will be spared from expensive clinical trials that may subject them to risk. Another approach would be to compare the effectiveness of IV LEV + normal saline with IV LEV + IV TPM. This design would be considered a placebo-controlled trial in which I would test for superiority instead. The advantage of conducting a noninferiority trial instead is the smaller sample size, especially if the difference in efficacy of IV LEV and IV TPM is small.

CHAPTER 5

CONCLUSIONS

Status epilepticus is a life-threatening neurological emergency defined as abnormally prolonged seizure (>5 mins) that can have long-term consequences including neuronal cell injury and rewiring of neuronal networks (Trinka et al. 2015). Despite having evidence-based guidelines for the management of convulsive SE, approximately 30% of cases fail to respond to first-line therapies and progress to more serious conditions with high mortality rates (Treiman et al. 1998; Logroscino et al. 1997; Vignatelli, Tonon, and Alessandro 2003). Further, up to 50% of established SE cases fail to respond to second-line therapies, and as high as 94% of refractory SE cases do not respond to a third treatment (Malamiri et al. 2012; Agarwal et al. 2007; W. B. Chen et al. 2011; U. Misra, Kalita, and Maurya 2012; Lyttle et al. 2019; Dalziel et al. 2019; Gujjar et al. 2017; Mundlamuri et al. 2015; Nene et al. 2019; Treiman et al. 1998). Thus, there remains an unmet need for more effective and safer drugs to better manage this condition at all stages.

The main **objective of my dissertation is to develop alternative therapies for seizure emergencies**. As part of my work, I provided a review of human and canine epilepsy and SE and discussed the translatability of research between the two diseases. My work in CNS-acting drugs spanned across the drug development pipeline and included the development of 1) ALLO for the treatment of SE, 2) LCM for the treatment of established SE, and 3) TPM for the treatment of established SE.

Allopregnanolone is a naturally-occurring neurosteroid that is a positive allosteric modulate $GABA_A$ receptors that has potential as an early treatment of SE. My working hypothesis is ALLO would be beneficial in the early treatment of SE based on its novel mechanism of action and ability to get into the brain quickly. The specific aims of my project were to characterize the PK, PD, and safety/tolerability following single doses of IV and IM ALLO in dogs. I found that following IV dosing, ALLO exposure increases proportionately with dose within the doses used in my studies (1-4 mg/kg). Behavioral responses and iEEG data illustrate the rapid onset of effect following IV ALLO administration which were dose-dependent. This information was used to determine the dose for a future efficacy study. A dose of 2 mg/kg infused IV over 5 minutes is predicted to result in plasma ALLO concentration well above the plasma EC_{50} but below levels associated with heavy sedation. IM ALLO has great potential to be useful as a first-line treatment for SE, but the current formulations do not attain high enough plasma concentrations to alter iEEG activity. Therefore, in order to continue IM ALLO development, alternate approaches to increased early drug concentrations should be explored. A limitation to IM dosing is that given the current formulation, administration of a larger dose within a small injection volume is not feasible. Alternatives to overcome this limitation include increasing the water solubility, using different administration strategies, and/or optimizing the IM formulation by adding multiple co-solvents and/or small volumes of an organic solvent. As a next step in the development of IV ALLO for the first-line treatment of SE, I designed a prospective, double-blinded, randomized non-inferiority study in

canine patients with SE to test my hypothesis that IV ALLO is just as effective as IV DZP. An alternative first-line treatment for SE will improve patient (canine and human) care by preventing progression to later, more severe stages of SE and critical care admission.

Lacosamide is an antiseizure drug that enhances the slow inactivation of voltage-gated sodium channels and has potential as a treatment for established SE. Its use, particularly in critically-ill patients, is limited by cardiac safety concerns. My working hypothesis is that IV LCM increases the risk for PR prolongation, especially in the critically-ill population. The specific aim of my project was to estimate the prevalence of PR prolongation in the critically-ill patient population following IV LCM administration. While I found no significant difference between the median pre- and post-dose PR interval, the prevalence of PR prolongation was estimated to be 8%, which is higher than the prevalence of 0.4% reported in ambulatory patients with epilepsy. I also found that the occurrence of PR prolongation following IV LCM administration is positively associated with age, the total daily dose of LCM, and serum potassium levels. This suggests there is a subpopulation of critically-ill patients who are at higher risk of PR prolongation. However, considering that these results are generated from a small number of events (n=7/88) without a control group, additional work should be conducted to verify my findings. For example, a prospective study to calculate the true prevalence of PR prolongation and identify clinical predictors of PR prolongation would be used to further help address concerns for its use in the critically ill

population and/or seizure emergencies. To address this need, I designed a prospective observational study of PR prolongation in critically-ill patients who have been administered IV LCM matched to a control group by age, admission date, and ICU. Developing IV LCM as an alternative treatment for established SE by characterizing the patients in whom it can be used safely (without concern for cardiac conduction disturbances) would improve patient care by preventing the progression to refractory stages of SE and the use of TLAs, which have an inherent risk of systemic complications on their own and increased risk for rebound seizures.

Topiramate is an antiseizure drug that potentiates GABA current and antagonize AMPA/kainite receptors and has potential as an adjunctive treatment for refractory SE. My working hypothesis is that IV TPM would be beneficial as an adjunctive treatment for established SE based on its multiple mechanisms of action and low potential for drug-drug interactions. The specific aims of my project were to characterize the PK, PD, and safety/tolerability following single doses of IV TPM in dogs. My major finding included IV TPM at 10- and 20-mg/kg was 1) safe and well-tolerated, 2) resulted in a statistically significant change in iEEG activity within 15 minutes of drug infusion, and 3) co-medication with PB was associated a 5.6-fold higher clearance. With these results, I determined a dosing regimen for a clinical trial design in CSE. In order to inform the design of clinical trial demonstrating safety and efficacy in human established SE, I designed a double-blinded, randomized, multi-center noninferiority study in

canine patients with SE who have failed three rounds of BZDs to test my hypothesis that IV TPM is just as effective as IV LEV. Another pathway to the development of IV TPM is as a temporary replacement for oral TPM therapy. Single-dose 100 mg IV TPM bioequivalence has been demonstrated, however the safety of higher loading doses and repeated doses of IV TPM is still unknown. To address these safety concerns, I designed an open-labeled single- and multiple-ascending dose study in patients stable on oral TPM. IV TPM as another alternative treatment for the treatment of established SE would improve patient (canine and human) care by preventing progression to refractory stages of SE and the need for TLAs.

The majority of my work was conducted in dogs with and without naturally-occurring epilepsy. Although studies conducted in animals are typically thought of as “preclinical,” I would like to emphasize that our animals are patients, too. The studies conducted resemble Phase I/II dose-escalation studies aimed at characterizing the PK, safety, and early markers of PD of a new compound in people/patients. As mentioned in Chapter 1.3.3, dogs have naturally-occurring epilepsy and SE with etiologies, pathology, and phenomenology that closely mimic the human disorder. The advantages of using canine SE as a translational platform for therapeutic research include the ability to evaluate behavioral responses, accommodate human devices, and estimate a response rate to potential therapies for human SE. Therefore, the results of my canine studies will provide, at the very least, considerations for clinical trial designs in people.

My research suggests that there are promising therapies in development for the management of SE, which will significantly improve patient lives by offering safer use of current antiseizure drugs or more effective therapies. There are many pathways into which these projects can take, including conducting clinical trials in dogs with naturally-occurring SE and single- and multiple-ascending dose studies in patients.

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